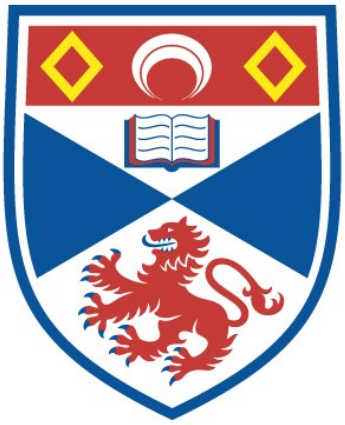
ENVIRONMENTAL PHYSIOLOGY OF TWO HOVERFLIES, 'ERISTALIS TENAX' AND 'E. PERTINAX'

Sabine Bressin

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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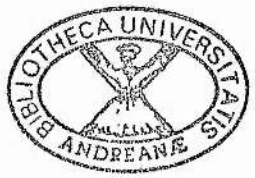

Declaration

I, Sabine Bressin, hereby certify that this thesis, which is approximately 75000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in October 1995 and as a candidate for the degree of Ph.D. in October 1996; the higher study for which this is a record was carried out in the University of St Andrews between 1995 and 1999.

Date 26/2/99 Signature of candidate

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Ph.D. in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

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A mes proches

To my little friend who kept me company
during the long hours of field work

Acknowledgements

So many people have helped me through this work, even if only with a kind word, it is hard not to forget anyone; to all of them I say thank you.

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Thanks to Simon, Harriet, Froggy, Liz (my office mates) and to many others in the Bute for their company and for providing stress relief. Isobel, assisted my survival by keeping the office spotless and sometimes transforming it in an organic grocery and essentially offering help whenever she could.

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Abstract

Eristalis tenax and *E. pertinax* are two closely related Scottish hoverflies that share various ecological features, such as feeding sources, but differ in others (e.g. only adult *E. tenax* overwinter). The physiological performances and behaviour of these flies were studied in relation to the hygrothermal constraints faced, as were the physiological adaptations in the two (summer and winter) generations of *E. tenax*.

Their water loss rates are rather high, lying in the upper part of the range for mesic insects. Rates are higher in *E. tenax* than in *E. pertinax*, and higher in male than in female *E. pertinax*. Water loss rates can be modified, notably in overwintering, water stressed females.

Because of the rather high levels of evaporative cooling, these flies equilibrate at a temperature lower than ambient temperature. This work supports Bakken's (1976) recommendation of using equilibrium temperature as the "external" temperature for the estimation of warming and cooling constants, and further analyses the effects of evaporative cooling on these constants.

Both species have endothermic abilities. Only *E. tenax* thermoregulate to a moderate degree in forward flight, but hovering male *E. pertinax* are excellent thermoregulators. Haemolymph shunting appears to be in operation in *E. tenax*, but is unlikely to be a controlled process.

Large flies exchange heat with the environment more slowly, have faster warm-up rates, and take off at (and fly with) higher thoracic temperatures than small ones. Both linear dimensions and mass have to be considered, as the various aspects of insects' biology are not affected by the same size factor and because only then can "real" sex differences be distinguished from ones resulting from a difference in shape in males and females.

In Scotland, male *E. pertinax* hover whereas male *E. tenax* do not. Hovering duration is strongly influenced by temperature. The foraging activity of these flies appears to be controlled in part by a circadian rhythm with additional effects of temperature in male *E. tenax*, and of light in *E. pertinax*.

Overwintering female *E. tenax* select crevices in caves and ruins that offer a relatively constant, warm and highly humid microclimate. The start and end of overwintering appear to be triggered by changes in ambient temperature.

Further opportunities for comparative studies of eristalines across their broad geographical range and between summer and winter generations are considered.

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Chapter 1 - Introduction

For over 2000 years, there has been some confusion between the honeybee and its supposed mimic, the syrphid drone fly *Eristalis tenax*. In the Middle East and the West, from early Egyptian, Greek, and Roman times, authors have perpetuated the myth that bees could be generated from the carcasses of oxen (Baron von Osten Sacken 1893, cited by Buckton 1895). Even earlier, the biblical Samson is said to have taken honey from the swarm of bees he found in the carcass of a lion he had previously slain (Judges xiv. 14.):

> "Out of the eater came forth meat;
>
> Out of the strong came forth sweetness"

However, it seems unlikely that bees would build combs in carcasses (Buckton 1895). Baron von Osten Sacken (1893) suggested that the superstition originated from the resemblance of the adult *E. tenax* to the drone honeybee. However, even this proposition is not quite satisfactory, as *E. tenax* larvae are adapted for an aquatic environment that would not be provided by a carcass, unless it was submerged, which does not seem to be the case in the myths (Buckton 1895). In addition, the honey found by Samson in the lion's carcass has still to be accounted for.

Certainly, various aspects of the natural history of *Eristalis*, including its famous resemblance to the honeybee, make it a very interesting subject to study. Much is known about the honeybee, far less about the drone fly; but if it has fooled so many observers it must share many aspects of its life history with the honeybee. Also, *E. tenax* is quite remarkable as it is one of the few insects overwintering as an adult in temperate parts of the world. It has to withstand the heat and dryness of summers as well as the coldness of winters; this implies varied behavioural and physiological regulatory mechanisms.

Eristalis pertinax belongs to same genus and looks very similar to *E. tenax*. The two species are often encountered together, but *E. pertinax* does not overwinter as an adult. Thus, the study of these two species might show interesting similarities and differences in their behaviour and physiology.

1.1 Natural history of hoverflies with special reference to *Eristalis tenax* (Linnaeus, 1758) and *E. pertinax* (Scopoli, 1763)

1.1.1 General points and description

A/ Background and distribution

Most of the information given in this section comes from two books: "British Hoverflies" by Stubbs and Falk (1983) and "Hoverflies" by Gilbert (1986). These are, to date, the most comprehensive books available on British hoverflies, discussing their natural history and providing keys and drawings for identification purposes.

Hoverflies are true flies (Diptera) belonging to the family Syrphidae, which is itself split into three subfamilies: Syrphinae, Eristalinae, and the rarer Microdontinae. *E.tenax* and *E. pertinax* belong to the subfamily Eristalinae, tribe Eristalini.

Buckton (1895) suggests that Linnaeus chose *Eristalis* as a generic name because the larva floating on water shows bluish and rosy iridescent colours; the species name *tenax* would refer to the tenacity of life observed for both larva and imago; Linnaeus had observed that some larvae, accidentally mixed into the pulp employed in a paper mill, survived the pressure of the rollers (Buckton 1895).

Over 5300 species of hoverflies, or syrphids, are known worldwide (Gilbert *et al.* 1985a); about 250 have been described in Britain (Stubbs and Falk 1983). Information from various sources indicates a wide distribution, from almost every part of the world. In particular, *E. tenax* and *E. pertinax*, are found in Western Europe, the Netherlands, Asia, Eastern Europe (Poland), in America, New Zealand, Africa and South Africa. Therefore, they seem to be able to cope with very different climatic conditions. *E. tenax* survives the very cold winters of Poland and other regions by

overwintering (Kendall and Stradling 1972, Moog and Ernst 1978, Siuda 1963). Both eristalines are active in humid temperate Britain as well as in hot arid Israel (Simon Potts pers. comm.) or Spain (Herrera 1990, 1988).

Hoverflies are not only found in climatically contrasting regions, but also in a variety of habitats, such as woodlands, meadows, coastal habitats, bogs, etc., and also in man-made environments such as gardens and parks (Stubbs and Falk 1983). The early stages of syrphids exploit numerous habitats as well. For example, they are seen in dead wood, sap runs, in the bulbs of *Narcissus* and *Iris*, fungi, rot holes, ponds, ditches, etc. Some larvae even develop inside wasps or ants nests, and some feed on aphids or caterpillars. Syrphids also impress by their variety; there is a 250-fold mass difference between the smallest and the largest (Gilbert *et al.* 1985a). As their name suggests, hoverflies are often seen hovering, usually near flowers.

B/ Morphology and appearance

Various morphological details provide easy means to identify adult hoverflies (Stubbs and Falk 1983). The thorax lacks bristles and is covered with fine hair. Hoverflies are the only Diptera to have a vena spuria, or false vein, on the wing. In addition, in contrast to most other Diptera, syrphids have two outer cross-veins running near the margin of the wing. The antennae are made of three segments, the most distal one bearing an arista (bristle-like structure) either dorsally or apically. The large eyes (probably to provide acute vision to these fast flying insects (Gilbert 1986)) meet on top of the head in most males (holoptic) but are always widely separated in females. Sexes are distinguished by the shape of the distal part of the abdomen. The last abdominal segments of male hoverflies are folded so that the genitalia are tucked underneath the abdomen, giving the abdomen end a rounded appearance. In contrast, the female abdomen end is gently tapering.

As in most *Eristalis*, the abdomen of *E. tenax* and *E. pertinax* varies from black to brightly marked orange/yellow. The two species look similar and are often seen as the same time. However, the two can easily be distinguished by a few characters: the front tarsi are black in *E. tenax* but red in *E. pertinax*; *E. tenax* has a conspicuous hair fringe on the dorsal and ventral surfaces of the distal half of the hind tibiae, a very broad black

face stripe, and two rows of dense hair across the eyes; in addition, the abdomen of *E. tenax* is more cylindrical than the slender and more gently tapering abdomen of *E. pertinax*.

Keys for identification of the earlier stages are given in Chandler (1968; cited by Gilbert 1986) for the eggs, and in Rotheray (1986), Dixon (1960), Hartley (1961), and Dolezil (1970), (all cited by Gilbert (1986)), for the larval stages. As this work is mostly concerned with adult hoverflies, the subject of identification of early stages will not be developed further.

C/ Polymorphism

E. tenax and the other hoverflies from the genus *Eristalis* show variation in the extent and brightness of their abdominal patterns and/or thoracic hair. The thoracic pubescence of *E. tenax* varies from nearly white to dark brown; the abdominal, from completely brown to mainly orange. The cuticular markings consist of a pair of semi-oval yellow or brown spots on the dorsum of tergites 2, sometimes extending to tergite 3 (cf. classification designed by Heal 1979a). In addition, *E. tenax* shows some variation in the coloration of the ventral surface of the abdomen, and of the hind femora.

The amount of phenotypic variation is not constant between species (Holloway 1993). Selection for mimicry plays a part in colour pattern variation, but this variation may also serve other functions such as thermoregulation; the relevance of colour to ectothermic warming (dark individuals absorbing radiation more quickly, and so warming up faster than light ones) has been suggested by many authors, and is reviewed in the next section. Hoverflies of the genus *Eristalis* have long flight periods and are multivoltine temperate species; it would seem advantageous for a developing insect to adjust its adult phenotype in some ways to variation in the environment (phenotypic plasticity). The evolution of colour patterns in *Eristalis* species may therefore result from a trade off between selection for mimicry and thermal effects. It seems that the balance between selection for mimicry and thermal effects varies between species.

Three factors have been found to influence such a variation:

- genes
- age
- temperature (mainly pupal development temperature)

The relative importance of these three factors varies from species to species. Heal (1982) showed that, in the laboratory, the pupal developmental temperature influences the shade of the morph of certain species; high pupal temperature inhibits the formation of melanin, thus lighter patterns are obtained. In the wild, it is difficult to observe the influence of pupal temperature because of the great mobility of these insects, but sometimes a slight mode change in darkness is recorded (Heal 1982, 1989; Holloway 1993). In some *Eristalis* species, laboratory breeding experiments have also demonstrated the genetic basis of pattern variation and its alteration with age (Heal 1989). The abdominal pattern of *E. tenax* is mainly controlled by a major gene locus with alleles for light and dark colour pattern (light being dominant) and, to a lesser extent, by environmental factors (pupal temperature) and adult age (see below). This results in a range of patterns within two principal categories (light and dark) (Heal 1979a). The thoracic hair darkness is also influenced by the pupal temperature. The same is true for the variation in colour of the thoracic pubescence of *E. intricarius*, but here the pupal temperature has a greater influence than in *E. tenax* (Heal 1979b). In contrast, in *E. arbustorum*, where females mimic small dark bees and males mimic wasps, almost all the variation is non-genetic; the sexual dimorphism is genetically determined, but the phenotypic variation within the sexes is attributable to pupal temperature in males and to age in females. This results in observable seasonal fluctuations in male phenotypes (Heal 1981).

Heal (1982) found a bimodal distribution in the summer and a unimodal distribution in the spring (females only) in *E. tenax* abdominal patterns; he proposed that this is the result of light individuals becoming darker and dark individuals lighter during overwintering (respectively secondary melanization and incorporation of pale pigments in the epidermis, below the cuticle). Heal suggested that this pattern could be linked to selection for mimicry; few brightly coloured models are around in spring, so it pays to be rather dark, whereas in the summer more brightly coloured models appear, and bimodality with many light patterns becomes a good strategy. Also, at high density of mimic, discrimination by predators could generate apostatic selection; more variation in the summer makes sense when the mimics are more abundant. In addition,

thermal considerations would encourage dark phenotypes in spring when it is cooler and lighter patterns in summer when there is a risk of over-heating.

E. tenax has not evolved a 'perfect' resemblance to honeybees. Heal (1982) suggested two reasons for that:

- the drone fly may get some protection from the partial mimicry of wasps by having a modified pattern; this could in part explain the occurrence of brighter patterns when wasps are around
- more effective thermoregulation ; a black stripe is present just above the dorsal blood vessel, which may serve to increase solar radiation absorption in a species that uses basking, (honeybees do not bask, and lack this stripe).

The genetics controlling the coloration in *A. mellifera* are the same as in *E. tenax;* much of the variation in both is attributable to corresponding genetic polymorphisms (Heal 1982). In addition, in the honeybee, the extent of the abdominal bands is also sensitive to temperature and varies seasonally (Soose 1954; cited by Heal 1982). In sympatric populations, both honeybee and drone fly have a similar range of abdominal patterns, and both have a bimodal distribution (in summer and autumn) (Heal 1982).

Eristalis species also exhibit sexual dimorphism in their colour pattern, with on average the females being darker than the males. Holloway (1993) argued that this dimorphism could reflect differences in the ecology of the two sexes; females would need to get a higher solar absorption as they spend more time in cool and damp places which are suitable for oviposition; they are also more mobile. In *E. arbustorum*, the previously described sexual dimorphism could have arisen because of differences in the requirements against predation of the two sexes: flying males look like wasps whereas foraging females mimic bees (Heal 1981). Thus, again both selection for mimicry and thermoregulation apply.

Holloway (1993) looked at the possible relationship between temperature and colour pattern in several species of the *Eristalis* genus, namely *E. arbustorum, E. horticola, E. nemorum,* and *E. abusisivus,* in the Netherlands. He found a good relationship in both sexes of all these species except in female *E. abusivus* and *E. nemorum,* paler insects being more abundant in warmer months. He also found that female but not male *E. arbustorum* collected inland were on average paler than those collected

on coastal areas (cooler areas). This seems in contradiction with Heal's findings (1981) (see above) where the darkness in females was proposed to be influenced mainly by age rather than environmental factors. However, Holloway suggested that the cause of this discrepancy was due to a far longer life of females in the laboratory than in the wild; field collections from both authors appear to agree. Thus, there seems to be a weak environmental influence on the darkness of female *E. arbustorum*.

The examples described above seem to support the view that colour pattern variation in hoverflies of the genus *Eristalis* results from a trade off between mimicry and thermoregulation. However, Holloway (1993) pointed out that if the relationship between colour and season in some *Eristalis* species has some adaptive function, it is curious that there is so much difference in the phenotypic plasticity of species that otherwise share a great deal of their ecology. In one example, an explanation for such a difference could be suggested: *E. intricarius* is hairier than *E. tenax* and is also more sensitive to pupal temperature, which makes sense because the danger of over-heating is probably greater in a dark insect that is also hairy (Heal 1982). Holloway (1993) suggested that more investigations should be carried out to establish that variation in colour is linked to thermoregulation.

D/ Mimicry

Some hoverflies are said to mimic (Batesian mimicry) wasps and bees, both in appearance and in behaviour; however the degree of credibility is sometimes low. It seems that the mimicry of honeybees, *Apis mellifera*, is quite common amongst flies active in spring, because neither *Bombus* workers nor *Vespula* workers, which are also potential models, are abundant until the summer. *Eristalis* spp. do not only mimic honeybees visually but also behaviourally, during flight and feeding; in addition, they produce a similar buzzing sound. *E. tenax*, or the drone fly, as its name suggests, looks like male honeybees (drones). This raises the question of the selective advantage gained by *E. tenax* in mimicking stingless, and thus harmless, animals. Morgan (1908, cited by Brower and Brower 1965) suggested that the resemblance of both drone bees and drone flies to worker bees (the stinging ones) outweighs any difference in details (colour pattern, size, etc.). He showed that young birds having

experienced worker bees will avoid drone bees and drone flies. These birds put all three insects within one "generic image"; they generalise from the experience with stings of workers and avoid all three insects. Brower and Brower (1965) raised another question about the evolution of mimicry in *Eristalis*. It is very likely that *Eristalis* preceded apiculture in North America; this would suggest that mimicry evolved without a model. Brower and Brower (1965) suggested that flies from the *Eristalis* genus have a general bee-like appearance; as honeybees and their mimics have similar feeding habits, this resemblance was then enhanced through selection for mimicry after the introduction of honeybees. Mimicry has also been questioned by other authors. Holloway (1976) suggested that the morphological and behavioural resemblance between drone fly and honeybee could be the result of convergent evolution in two species having similar food gathering requirements; like their model, drone flies feed on flower nectar and pollen and, sometimes, on honeydew secreted on plant leaves by aphids (see below). Thus, this resemblance is not necessarily the result of mimicry. Whittington (1994; personal communication), while studying African eristaline mimicry, did not find the selective pressure to drive the evolution of this mimicry system. Several natural predators would need to be recognised to suggest that the drone fly derives benefit from resembling honeybees. Moreover, there exist numerous specialist bee predators against which mimicry could not offer any protection, but quite the contrary. Also, Beddard (1892, cited by Brower and Brower 1965) showed that at least three species of lizard happily feed on the flies. However, in some instances some protection seems to be gained; Brower and Brower (1965) demonstrated that young toads exposed to stinging worker bees subsequently take fewer drone flies than control animals that have not been exposed. The authors also showed that it is the sting that is the source of noxiousness, as fewer honey bees whose stinging mechanism had been removed were rejected by the toad than intact ones. In this series of experiments the importance of behaviour in mimicry was also demonstrated; decreasing the buzzing sound (by removing the wings) of both drone flies and honeybees led to less insects being rejected by the toads. Audio mimicry acts in addition to visual mimicry. Visual mimicry can be isolated by suppressing the buzzing sound (dead specimens); the toads do still avoid drone flies after

experience with honeybees (Brower and Brower 1962). Brower and Brower's laboratory work shows that protection might be gained in some instances, but it still does not mean that mimicry is actually at work; a natural selective pressure has still to be found. In addition, Whittington (1994) observed *E. tenax* trying to enter a honeybee nest. This particular fly did not succeed but it is not known if drone flies usually tend to enter bee nests (they could do so to steal some honey). If that were the case, mimicry could possibly offer protection to the fly from the honeybees themselves. Buzzing (sound mimicry) could also assist. It is not known if chemical mimicry occurs in eristalines. However, mimicry may be of low importance for the purpose of entering bee nests, as *Volucella bombylans* (a bumblebee mimic) enters nests of both models and non-models. Resemblance (behavioural and morphological) certainly exists between drone flies and honeybees but the existence of a true mimicry system is still controversial.

1.1.2 Life cycle

Being flies, syrphids develop from eggs and pass through the larval and pupal stages before emerging as adults. These are described in Gilbert (1986). The duration of these developmental stages varies between species, from less than two weeks to up to five years, and within species, being influenced by external factors such as temperature.

Eggs are laid in or close to potential larval food, the females probably being attracted by odour. For example, female *E. tenax* and *E. pertinax* are attracted by the smell of dung, stagnant water, decaying matter, etc. The eggs are ovoid and their size generally reflects the size of adults. The egg surface is sculptured, a character that provides a means for identification. Eggs hatch rapidly, but nevertheless the duration of the egg life is influenced by both temperature and humidity. The size of the batches is generally characteristic of the larval feeding habit. *E. tenax* and *E. pertinax* lay batches of up to two hundred eggs, reflecting the filter feeding habit of the larvae from water containing plenty of food. In contrast, species with aphid-feeding larvae lay eggs in small groups, as the food in one particular site is limited.

Larvae can be divided into two major categories according to their feeding habits:

* carnivores: obligate, feeding on aphids only; or facultative, which can, in addition, use rotting plant material. These larvae are coloured by blood pigments and fat deposits.
* others: these are white. This category can be further divided into three groups:
    - those larvae that feed on plant tissues and plant products
    - those that live in the nests of social insects
    - lastly, those that scavenge on, or filter, decaying matter.

Eristalis larvae, 'rat-tailed maggots', belong to the last group; they are aquatic and are found in drains, ponds, farm yard manure, sewage, etc. The 'tail' consists of three telescopic tube-like sections; it is used for breathing air by the submerged larva. Eight hydrophobe, oiled feather-like hairs at the distal end prevent water from entering the tube. The mouthparts are well adapted to filter feeding with one coarse and one fine filter being present. A muscular pump draws water in and ejects it out, cleaning the filters. Osmoregulation is effected by rectal gills which actively take up salts from water.

Larval life is completed after three instars (with moulting between each); the length of the larval life is influenced by various factors such as temperature and the seasonal timing of the appearance of adults. Some species overwinter as larvae, thus having a long diapause. The pupation stage follows. Mature *Eristalis* larvae crawl out of water to pupate. As this stage occurs in the unshed hardened skin of the third instar, it is called a 'puparium' rather than a pupa. Normally, this stage is quite short, but it can be very much extended if the species overwinters as a puparium.

Adult males tend to emerge earlier than females: being smaller, they develop faster. As a consequence, they have time to mature and are ready to mate when the females emerge. The teneral fly is pale (colour and markings appear after several hours), and the cuticle is soft; the first few hours of adult life must be spent immobile to allow the cuticle to harden and the wings to unfold. For this reason, emergence occurs in the early morning, decreasing the risk of being preyed upon without a chance of escaping. The abdomen is expanded by swallowing air.

Most species of hoverflies are multivoltine (several generations per year); the number of generations depends on climatic conditions and food availability, but Gilbert (1986) suggested that three generations a year is

probably the maximum. Some hoverflies, including some *E. tenax* populations, emigrate to southern Europe for the winter. Of the ones that stay in Britain, most spend the winter as larvae or pupae; and a few, including *E. tenax* and *Eristalinus aeneus*, overwinter as adults. In late autumn, female *E. tenax* of the last generation before the winter (the autumn generation) are inseminated with sperm (which is stored); they then retreat into sites such as caves, rock crevices, or old buildings to spend the winter. It seems that certain levels of humidity (Kendall and Stradling 1972) and darkness are required for a site to be suitable for overwintering. However, sites where flooding is a risk are not acceptable (Kendall and Stradling 1972). Also, Siuda (1963) and Moog and Ernst (1978) suggested that light intensity has some importance on the choice of the hibernating places; *E. tenax* are found not too far from the entrance of caves. Frequently, the flies cluster together in the same crevice (Callieri 1994, Kendall and Stradling 1972, Moog and Ernst 1978, Siuda 1963). Some individuals are active at times during the winter, probably if they have access to food, and maybe to replenish their water reserves (see the next sections). Although the majority of overwintering drone flies are females, some males do also spend the winter as adults (Kendall and Stradling 1972). In spring, the first adults of *E. tenax* to emerge are actually "old" females which have overwintered. The eggs are fertilized, laid, and a new generation develops.

Apart from 'old age', hoverflies can die for a number of reasons. Earlier stages, essentially larvae feeding on aphids, are susceptible to parasitism by wasps which lay their eggs inside the larvae (Gilbert 1986). Parasites of adults seem rare, but fungi of the genera *Entomophthora* and *Empusa* are responsible for some deaths; hoverflies are also preyed upon by a number of animals such as birds, toads, wasps, etc., and by carnivorous plants (for example the sundew, *Drosera rotundifolia*) (Gilbert 1986). Siuda (1963) reports that the spider *Tegenaria domestica* preys upon overwintering female *E. tenax*. Certainly, dead drone flies are often found entangled in spider webs in overwintering sites (pers. obs.). To some extent adult hoverflies may be protected from predators by their resemblance to wasps or bees, but attacks by wasps are often witnessed (pers. obs.). One such attack was observed. A female *E. tenax* was attacked by a wasp. The two insects fell on the ground. The fly buzzed and

struggled, but the struggle stopped after a second wasp joined the fight. After having overpowered the fly, the wasps proceeded in cutting their prey into pieces. After about 10 minutes, one wasp left with the thorax. The other wasp seemed to struggle with another piece of fly but left without carrying anything. The abdomen, legs, wings and head of the unfortunate drone fly were found scattered on the ground.

1.1.3 Activities

As in most other insects, the various activities of syrphids are very much dependent on factors such as temperature, light, wind, and humidity (see Section 1.5). Thus, these flies have diurnal activity patterns (Gilbert 1985b). Although some species are crepuscular (e.g. *Copestylum vesicularium* (Gilbert 1985b)), hoverflies are essentially day-flying insects.

A/ Feeding

Hoverflies feed on nectar, pollen, and honeydew. Hoverflies participate in pollination as a result of the pollen getting stuck to their hairs. For example, Jarlan et al (1997) have found that *E. tenax* is a potential powerful pollinator of sweet pepper. Mostly composite, small labiates, umbiliferous and rosaceous flowers are visited, but grasses and plantains are not ignored, and, in the autumn, ivy (*Hedera helix*) blossoms attract many hoverflies. Nectar is almost a pure solution of sugars (Proctor et al 1996, Corbet et al 1979, Sotavalta et al 1962) which are rapidly broken down to yield energy. In contrast, pollen contains some proteins and lipids, and some sugars rich in energy (Proctor et al 1996). Thus, weight for weight, nectar provides more energy, faster. Males that hover have been found to have their abdomen partly filled with air, probably to reduce their mass and save energy, and tend to feed on nectar only (Gilbert 1986). Pollen is usually eaten by both males and females at the beginning of their life, because adults emerge with immature reproductive organs and gametes: the pollen proteins are essential for maturation. Later, pollen is mostly consumed by females which need it for egg maturation (Gilbert 1986). However, some species (for example, *Platycheirus clypeatus*) do eat only pollen throughout their life (Gilbert 1985c).

The insects' mouth parts are usually adapted to the kind of food they have to deal with. Long, slender mouthparts and short labellar hairs usually go with a predominantly nectar diet; broad mouth parts with longer labellar hairs are found in those species that specialise in pollen feeding (Gilbert 1981). The morphology of the mouthparts of *E. tenax* suggests that this species takes both resources, but takes more nectar than pollen. Gilbert does not describe *E. pertinax*'s mouthparts, but as this species often feeds alongside *E. tenax*, it is probable that both species have similar mouth parts. *E. pertinax* has certainly been observed taking both nectar and pollen in the field (pers. obs.). In *E. tenax*, pollen collected by the mouth parts is not ground up, but is ingested intact. The flower's anthers are held between the labella which are then rubbed together. Holloway (1976) described the pollen feeding behaviour of *E. tenax*, which is covered with hair, and compared it with that of *Melanostoma fasciatum*, a sparsely haired hoverfly. She concluded that the drone fly takes pollen directly from entomophilous flowers' anthers, but also cleans off and consumes material that adheres to its body hairs. Holloway described how the pollen grains are combed off the hairs by the front and hind tibiae, and are transferred to pollen retaining bristles on the front and hind tarsi. Pollen is eaten from the front tarsi; grains from the hind tarsi are transferred to the front tarsi during leg scraping movements. In contrast, *M. fasciatum* seems to ingest pollen mostly directly from anemophilous plants. Few grains adhere to the sparse hair, and when these are combed off, they are not retained by the bristles on the tarsi. Holloway went on to suggest that, in most syrphids, pollen feeding has been supplemented and, in some species, probably replaced by pollen-collecting systems operating as the fly collects nectar; these systems make pollen gathering a more efficient system, as the time required for foraging is reduced.

However, Gilbert (1981) did not agree with this claim. He observed that only a few species (e.g. *E. arbustorum*) regularly carry appreciable amounts of pollen. Also, cleaning occurs whether there is pollen attached to body hairs or not, and many species are seen to reject cleaned material. Heavy pollen loads could depend on the flower visited; *Ranunculus*, used in Holloway's experiment, would cover its visitors with pollen, and this might have influenced the results. In addition, it seems that the palynophilic hairs on the front femora of *Platycheirus* species, which

Holloway thought important in pollen gathering, do not occur in females; yet, females feed more on pollen than males. Therefore, the controversy as to pollen gathering from body hairs in *E. tenax* remains. Certainly, during the present study, this species was not observed carrying much pollen (pers. obs.).

Gilbert (1981) found that larger hoverfly species take more nectar than pollen in their diet. Larger species need more energy in absolute terms than do smaller species. Gilbert (1981) suggested that pollen cannot provide energy fast enough to the large syrphids; thus, the need to consume nectar. He also suggested that pollen-feeding preceded nectar-feeding in hoverflies, because early syrphids (about 70MA) had short mouth parts adapted for that task. Gilbert (1981) also described how *E. tenax* feeds on sugar solution from capillary tubes. The proboscis is extended to the nectar surface; the labella separate and nectar is pumped in (muscular pump in the pharynx); as the nectar level decreases the labella gradually close (increase the capillarity) until no more nectar can be obtained; then, the proboscis is extended further. In the field, it has been shown that hoverflies can dissolve concentrated crystalline nectar on the surface of the nectaries by regurgitating crop contents or spitting saliva (Willmer 1983).

Feeding occurs from a wide variety of flowers. Nectar is secreted by glandular structures, the nectaries, found at the base of the ovary, usually deep in the flower corolla. The accessibility of nectar depends on the shape of the flower and on the length of the proboscis; nectar can only be obtained if the corolla is not much deeper than the length of the extended proboscis. Thus, for a particular fly species, the choice of flowers will, in part at least, depend on the depth of the corolla (Gilbert 1981). However, flower choice is also made according to the nutritional value of the pollen provided by the flower and according to the colour of the petals. *E. tenax* and *E. pertinax* seem to specialise on yellow and white flowers such as yellow chrysanthenums and ivy. Kay (1976) showed that *Eristalis* spp. have a stronger preference for the yellow morph of the wild radish (*Raphanus raphanistrum*) than for its white morph. Ilse's (1949) work on the spectral sensitivity of *E. tenax* suggested that *Eristalis* spp. may have trichromatic vision and be sensitive to UV reflectance, as in bees. Discrimination seems due to differences in wavelength rather than

differences in the degree of lightness. Bishop and Chung (1972) agreed with Ilse that the retina of *E. tenax* contains more than one photopigment; they suggested up to four. They also found evidence for the convergence of visual sensory capabilities of the drone fly and the worker honeybee; it is to be expected, as model and mimic (assuming mimicry) feed on the same flowers, that selection pressures have acted on the visual sensory capabilities of the drone fly to allow it to visit the same flowers as its model. Lunau and Wacht (1994) showed that freshly emerged imago *E. tenax* innately extend their proboscis in response to light stimuli falling in a range of wavelengths from 520 nm to 600 nm (yellow-green). Wacht et al. (1996) showed the same preference in various eristalines, including *E. pertinax*. They also demonstrated that water- soluble chemical constituents of pollen stimulate taste receptors in the labellar taste hair of *E. tenax*; this could be involved in pollen detection.

B/ Territoriality and mating

Flowers provide food, but also, in many cases, a site for male courting activities. Females are rarely seen hovering as most of their energy is needed for investment in eggs; males, however, (as for example male *E. tenax* and *E. pertinax*) are often seen hovering above flowers where females are feeding, courting them. Wellington and Fitzpatrick (1981) found that, in Canada, male *E. tenax* spend their adult life in a restricted area and can maintain territoriality. An interesting point is that the autumn generation does not seem to be territorial (Wellington and Fitzpatrick 1981). Whereas male *E. pertinax* seem to be territorial (pers. obs.), there is not much evidence that this is the case in *E. tenax* in Britain (Gilbert 1986, pers. obs.). This question of territoriality obviously requires more investigations. Wellington and Fitzpatrick described how Canadian male *E. tenax* spend their lives within a home range which includes sheltering, resting, basking, grooming, feeding, and defended mating sites (territories). The various activities are separated in time and space; for example, the males leave their territories to carry out functions such as feeding and resting. Territorial duties seem to be restricted to about 15 minutes at a time, and alternate with resting, basking, grooming, and feeding. Male *E. tenax* behave aggressively towards any insect other than females of the same species that intrude in the territory. If the intruder is a

female, mating might occur after both male and female have hovered in tandem (Wellington and Fitzpatrick 1981). Male drone flies search for and court females near flowers; they hover above the females while these feed (Gilbert 1986).

Sometimes, male hoverflies (for example *E. pertinax*, pers. obs.) hover not above flowers, but in one position, usually a sun spot, and dart away to chase passing insects that could be females of their own species, returning to the same spot if unsuccessful. However, not all species engage in hovering. In some, the males wait perched on twigs and chase any insect flying by. In other species, for example *Syrphus ribesii*, the males swarm and it is the female that chooses its mate within a swarm.

Mating can last from a few seconds to hours. The eggs are not fertilised immediately, but are stored in the female spermathecae. The sperm is nourished by the secretions of the female's special tube glands. These glands are particularly well developed in species such as *E. tenax* that overwinter as adults. In that case, the sperm is nourished throughout the winter, and eggs are only fertilised in the spring (Gilbert 1986).

1.2 Thermal biology in insects

1.2.1 General points

May (1979) defined thermoregulation as the maintenance of the body temperature (T_b) relatively independently of the environment, by means of behavioural and physiological responses. The maintenance of the body temperature at a certain level is important for the normal functioning of many processes. Thermoconforming, on the other hand, involves having a body temperature that parallels ambient temperature (T_a).
Thermoregulation involves two mechanisms; either changes in heat exchange with the environment brought about by behavioural and/or physiological strategies, or changes in metabolic heat production (May 1979, Willmer 1982a).

Insects are predominantly ectotherms, gaining heat from the environment, and regulating this heat gain mainly by behaviour. Heat is exchanged between an animal and its environment by radiation,

convection, conduction, and evaporation. It seems that for ectotherms, heat is principally gained by radiation (Parry 1951, Shepherd 1958, Willmer 1982a), and lost by convection (Digby 1955; Edney 1971; Bursell 1974; May 1979, 1985; Willmer 1982a). Conduction is limited by the small surface of contact between an insect and the substrate, and evaporation is very often avoided because of water stress (Willmer 1982a). Ectotherms control their body temperature mainly by behavioural means such as basking, taking various postures, burrowing, and seeking appropriate microhabitats; but they may also use some physiological mechanisms, such as evaporative cooling (e.g. Heinrich 1980a & b; May 1979, 1985; Willmer 1982a).

Endotherms employ the same means as ectotherms to regulate their body temperature, but in addition, they also rely on metabolic heat production. Most insect endotherms use endothermy prior to or during some activities only (for example to warm up before flight) (e.g. Casey 1989, Heath et al 1971; May 1979, 1985; Heinrich 1974, 1993; Heinrich and Esch 1994): they are therefore better described as heterotherms or "facultative endotherms".

Therefore, it should be noted that thermoregulation is not necessarily linked with endothermy. Insects might use endothermy to warm up before becoming active, but do not necessarily thermoregulate while they are active.

1.2.2 The effect of size and shape on thermal balance

An important point to consider about insects is their size. Most insects are small, and their thermal balance is very much affected by this fact. A small size means a small thermal mass and a large surface area to volume ratio. Surface area is related to the square of linear dimensions, whereas volume is proportional to their cube. Therefore, of two similarly shaped objects or animals, the small one has a larger surface relative to its volume than the big one (Schmidt-Nielsen 1990). In addition to size, the shape of the animal must be taken into account, as a flattened object has a larger surface area to volume ratio than a rounded object of the same mass.

The surface area to volume ratio is a physical parameter that is at the origin of many aspects of animal biology. A large surface area to volume

ratio means relatively more contact with and influence from the environment (Schmidt-Nielsen 1990). Having a large surface area to volume ratio and a small thermal mass means that an insect's body temperature does not remain stable, but follows changes in ambient temperature. In contrast, a large body with a large thermal mass and small surface area to volume ratio does not see its temperature altered by rapid changes in its environment. Only sustained changes in ambient temperature will affect its temperature. Likewise, small insects with a large surface relative to their volume will lose the heat they produce metabolically much more rapidly than larger insects. Thus, an animal with a large surface area to volume ratio will be less thermally stable than one with a small surface area to volume ratio (Casey 1989, Willmer 1982a, Withers 1992). This is well illustrated by the negative relationships that exist between passive warm-up and cooling-down rates and the insects' size that have been recorded by many authors (e.g. Bartholomew 1981, Bartholomew and Epting 1975, Gilbert 1984).

Therefore, in small animals, changes in body temperature can be very fast and can be triggered by even small differences in air temperature. Because they are affected by small and rapid temperature changes in the environment, when considering the thermal environment of an insect, we are really looking at the microenvironment, within a few millimeters of the animal (Willmer 1982a). For example, very sharp temperature gradients are observed above a heated surface; an insect moving through these gradients will experience rapid changes in its body temperature (May 1985, Willmer 1982a). In addition, heat losses are also very much increased by air movements/winds. This is particularly true for small animals because of their large surface area to volume ratio and it markedly affects the thermal balance of flying insects.

But being small also means being able to exploit microhabitats to control the body temperature. The environment of most terrestrial insects is thermally heterogeneous: insects can select very small parts of their habitat that offer the best environmental conditions (May 1985, Willmer 1982a). They can exploit the microenvironment created by small cracks in rocks, burrows, etc. They can take advantage of the temperature gradients created above heated surfaces to thermoregulate (May 1985, Willmer 1982a). Thus, they are able to thermoregulate using behavioural means.

For example, in hot deserts, some cicindelid beetles burrow to find cool and humid places (Dreisig 1980); while some insects such as dragonflies (May 1978) seek shade when ambient temperature increases. Overwintering female *E. tenax* spend the winter in crevices in rocks and probably exploit the stable microenvironment available in this habitat.

Yet, although insects are in general considered to be small, they do have a wide range of sizes. Obviously, the thermoregulation of a 2 mg fly is going to be different from that of a 2g Sphinx moth. It is now accepted that, everything else being equal, larger insects can attain a higher temperature excess (difference between body temperature, usually thoracic temperature, and air temperature) but take longer to reach it (Bartholomew 1981, Digby 1955, Heinrich 1993, May 1976, Willmer and Unwin 1981). Thus, larger insects can become active at lower ambient temperatures. However, when active at high temperature, they are more prone to overheating because heat is less easily dissipated. Small insects, on the other hand, warm up quickly, but require a higher ambient temperature to reach a sufficient body temperature to become active. Moreover, when they fly, air movements dissipate most of the heat produced, reducing the risk of overheating. Thus, in flight at low ambient temperature, small insects have many more difficulties in maintaining an adequate thoracic temperature for flight muscle activity (Casey 1989, Gilbert 1984, Heinrich 1993, May 1979, Willmer 1982a). *E. tenax* and *E. pertinax* , weighing around 100-200 mg, fall in the middle of the size range. Thus, it would be expected that they would be active at fairly moderate ambient temperatures, but they might be prone to overheating.

A very effective behaviour regarding thermal balance is the aggregation of insects, especially those living in colonies. Aggregation increases the thermal mass and decreases the effective surface area to volume ratio of the insects. Heat is retained better, and the maintenance of a local microclimate makes the animals less susceptible to environmental instability (Willmer 1982a). The effect of clustering has been clearly demonstrated in honeybee colonies. Davenport (1992) stated that the temperature in *Apis* clusters reaches 20 °C in early winter and 32-35 °C later on when breeding starts, this being much higher than ambient temperature. He calculated that a cluster of 10000 bees would reduce heat losses by 95.2% (Davenport 1985). *E. tenax* overwintering in crevices are

found in clusters, but usually of a few individuals (up to about 20 individuals, pers. obs.), although, sometimes, isolated individuals are found. Thermal considerations (and water regulation) could be involved in the clustering.

For those insects that can achieve endothermic warm-up, the relationship between warm-up rate and size (i.e. body mass) has been somewhat controversial. In heterothermic vertebrates, the increase in metabolic rates per unit mass of tissue with decreasing body mass more than compensates for the increase in heat loss: the rate of warm-up is negatively correlated with body mass (Bartholomew 1981). May (1976) predicted that in small insects heat losses would be the most important factor and that the relationship would be reversed. Stone and Willmer (1989), using species of Apoidea (bees), confirmed the prediction made by May (1976) that for very small insects, warm-up rate correlates positively with body mass. This relationship depends on the thermogenic abilities of the tissues and on heat losses.

1.2.3 The effect of insulation and colour on thermal balance

Insulation in insects can be provided by air sacs under the cuticle (dragonflies), pile, scales, hair or fur. It helps keep the heat inside the body, but at high ambient temperature it can also be an inconvenience because of the increased risk of overheating (e.g. Heinrich 1993, May 1985, Willmer 1982a).

Also, colour (or reflectance) has an effect on the thermal balance of insects. Digby (1955) suggested that, in temperate regions, the effect of colour should not be very important for the thermal balance of insects, because even very pale insects still absorb at least 75% of the received radiation. Nevertheless, some desert beetles reflect up to 74% of the radiation; this could be a crucial factor in avoiding overheating. Willmer and Unwin (1981) showed some effect of colour on the rate of change of body temperature, particularly on larger insects: darker forms warm up faster than lighter ones. In addition, some species of insects exist in different pigmented morphs (e.g. Kingsolver and Wiernasz 1991). The darker morphs (for example in butterflies) have been shown to reach a higher body temperature than the lighter morphs (Watt 1968, 1969, Douglas and Grula 1978, Willmer 1982a). Also, some insects have the

ability to change colour so as to alter their surface reflectance. For example, May (1978) found that the blue areas of the dragonflies *Lestes rectangularis* and *Enallagma civile* darken reversibly when ambient temperature falls below 15 °C, presumably allowing the absorption of more solar radiation.

E. tenax and *E. pertinax* only have a short pile covering their thorax and are thus not particularly well insulated. The various colour morphs of these eristalines could influence the thermal balance. Possibly, darker individuals, by absorbing more solar radiation through basking, could be active at lower ambient temperatures than paler ones. Alternatively, pale flies could be at an advantage when foraging during very hot periods, as they would reflect more solar radiation and be less prone to overheating. Such an influence of coloration has been suggested by Heal (1981) as has already been mentioned in the polymorphism section of this chapter.

1.2.4 Endothermy in insects

Some insects are able to raise their body temperature at low ambient temperature before they engage in certain activities either by relying on external heat or by endothermic warm-up or both. Because of their small size, it would be too costly for insects to maintain a high body temperature; but a small size allows rapid heating and cooling. Thus, heterothermic insects tend to use endothermy when they need to warm up rapidly for certain activities (like flight) and they cannot rely on behavioural means to achieve it. When they do not need to be active, they let their body temperature fall to approximately ambient temperature (Heinrich 1974, 1993). Warming up before flight by 'shivering' is a classical example of endothermy (e.g. Krammer 1970; Heinrich 1974, 1993; Heinrich and Esch 1994; May 1985). A striking example of endothermic warm-up is the one demonstrated by Heinrich (1987) in Noctuid moths of the subfamily Cuculiinae. These moths fly in winter, maintaining a thoracic temperature of 30-35 °C at ambient temperatures as low as 0 °C. To achieve an adequate thoracic temperature for flight, they warm up endothermically prior to taking off.

Heat can be generated by 'shivering' but also by the 'futile cycling of substrates'. Shivering involves the simultaneous contraction of antagonistic flight muscles, leading to the production of heat with no, or

very slight, movement of the wings. In insects, most of this metabolic heat is produced in the flight muscles. Flight muscles represent a substantial proportion of the body mass of a pterygote insect and can thus produce large amounts of heat. The flight muscles of syrphids make up about 15% of the total body weight (Gilbert 1986). Futile cycling of substrates (Newsholme and Crabtree 1973, Surholt and Newsholme 1981, Stone and Willmer 1989) generates heat by the non-productive flux of substrate through a series of anabolic and catabolic reactions. The substrates are interconverted, and the associated splitting of ATP molecules yields heat. One such possible cycle is the fructose-6-phosphate/fructose-1,6-diphosphate cycle (Davenport 1992, Newsholme and Crabtree 1973, May 1985, Surholt and Newsholme 1981).

Endothermy has traditionally been studied in large moths, bees, and beetles. As most flies are small, their use of endothermy is much less likely (because of increased heat loss rates). However, recent studies have demonstrated that some dipterans can use metabolically generated heat to warm up. Chappell and Morgan (1987) showed that the tachinid flies *Nowickia nitida* and *N. rostrata* increase their thorax temperature before flight by basking, whenever this possibility is available, but also by endothermy, if necessary. As a result, these flies can be active at low ambient temperature (5 - 8 °C). Morgan and Heinrich (1987) investigated the thermoregulation of bee- and wasp-mimicking syrphid flies. Many wasps and bees use endothermy, and their syrphid models often forage at the same time, on the same flowers; hence the interest in studying the endothermic capabilities of the flies. In this study, the flies were smaller than their models, the largest being the same size as the smallest bumblebees and wasps. The authors found that all the syrphids tested demonstrated a marked ability for endothermy. During foraging, the flies, using a combination of basking and endothermy, maintained a high thoracic temperature, just a few degrees below that of their models. Amongst the syrphids tested, Morgan and Heinrich included one fly of the *Eristalis* genus, *E. barda*, a *Bombus* mimic, which has an average mass of 115 mg, similar to that of *E. tenax* and *E. pertinax*. In an earlier study, Heinrich and Pantle (1975) demonstrated that small syrphids (mass below 30 mg) of the genus *Syrphus* maintained a high thoracic temperature by a combination of shivering and basking, while they congregated at leks.

Some non-mimetic syrphids (*Sericomyia lata* and *Eristalis* sp.) could also maintain thoracic temperatures at high levels during activity. Thus, it seems that maintaining a high thoracic temperature is not a special adaptation for mimicry in syrphids, but is necessary for the flies to be active; rather, it could have been a pre-adaptation for the evolution of mimicry in syrphid flies (Morgan and Heinrich 1987).

1.2.5 Thermoregulation during activity

Some insects are able to maintain their body (or part of their body, usually the thorax) at a temperature that is relatively constant during activities such as flight, feeding and walking. For many, the maintenance of a thoracic temperature within a narrow range is essential for optimal flight muscle performance (Coelho 1991, Heinrich 1993). This ensures that they can remain active at a wide range of ambient temperatures. Two problems have to be faced: keeping a body temperature high enough when ambient temperature is low and avoiding overheating when ambient temperature is high. Usually, it is the thorax that is thermoregulated because it contains most of the muscles an insect uses when active. Basking and/or endothermy allow the insect to elevate its body temperature to become active. When the insect becomes active, heat generated by the muscles tends to raise body temperature further, whereas the air currents created increase convective cooling and tend to lower it. Depending on the importance of these two processes, the equilibrium temperature reached can be adequate for the activity to continue, can be too low or can lead to the risk of overheating. Therefore, at low ambient temperature, the heat produced endothermically has to be retained in the thorax. At high ambient temperature, the problem of overheating can be overcome by actions such as retreating to a cool place (Hadley 1970, Dreisig 1980) or by avoiding being active at peak temperatures (Willmer 1982b, 1983).

Physiologically, an insect can either decrease its heat production or increase its heat loss (e.g. by the internal redistribution of the heat generated or the increase of evaporative cooling) when it risks overheating while active (May 1995, Heinrich 1993, Willmer 1982a). There has been some controversy as to whether insects are able to alter metabolic heat production during flight. Many authors support the idea

that thoracic temperature regulation in flying insects is achieved by the variation in evaporative and/or convective heat loss (Casey 1989, Heinrich 1993, Heinrich and Esch 1994). Heat production has been shown to be independent of ambient temperature by several workers (e.g. Heinrich 1974, Casey 1976, Joos et al 1991, Kammer 1981). For example, Joos et al (1991) found that the wing beat frequency of foraging bumblebees is primarily determined by morphometric characteristics related to lift requirements and is not correlated with ambient or thoracic temperature. Thus the findings of many authors (e.g. Casey 1976, 1989; Heinrich 1974, 1983, 1993; Heinrich and Esch 1994; Joos et al 1991) do not support thermoregulation by wing beat frequency variation. However, Harrison et al (1996) suggested that honeybees do thermoregulate in flight by varying metabolic heat production. May (1995) also reported that the wing beat frequency of the dragonflies *Anax junius* decreases with temperature. Roberts et al (1998) made a similar claim for the bee *Centris pallida*. They found that in hovering males, which are exceptionally good thermoregulators, wing beat frequency decreases with ambient temperature. They could discount haemolymph shunting and increased evaporative cooling as being the physiological processes involved in thermoregulation in this case. However, they recognise that the decrease in wing beat frequency with increasing ambient temperature may be an active physiological means of thermoregulation, but that it may also be the result of thermal effects on flight motor properties, such as an increased elastic storage of energy by the cuticle or the muscles which would give higher efficiencies at high ambient temperature. Thus, the controversy is still open, but it seems likely that thermoregulation in insects can be achieved by both varying the heat exchange with the environment and by altering metabolic heat production.

Heat loss might be increased by evaporative cooling, but only those insects that do not face any restriction in their access to water supplies can rely on this. For example, Heinrich (1980a and b) showed that honeybees extrude some fluid and evaporate it from their mouth parts when thermally stressed. This has been confirmed by Cooper et al (1985) who found that the proportion of honeybees returning to the hive with a droplet extruded increase from almost none at 20 °C to 40% at 40 °C. These authors noticed that the thoracic temperatures of pollen foragers is

significantly higher than that of nectar foragers at an ambient temperature of 40 °C, and that pollen foraging decreases during the hottest periods.

Heinrich and Buchmann (1986) have demonstrated another way to increase heat losses at high ambient temperature. Working with carpenter bees, they propose that these insects fly faster at high temperature so as to increase convective cooling. Ellington et al (1986) confirmed that flight metabolism is independent of flight speed. Thus, the increased cooling obtained by flying faster would not be offset by extra heat produced in order to fly faster (see also Heinrich and Esch 1994).

Heat can also be transferred by the haemolymph from a region at high temperature to a region at low temperature, where (assuming an uninsulated surface) it will be lost to the environment. The abdomen is usually cooler than the thorax in flight as the muscles that produce heat are found in the thorax. Moreover, the abdomen of insects is usually less well insulated than the thorax (at least some parts of it, e.g. the ventral surface in bees). The two eristaline flies studied here have the thorax covered with short pile, but not the abdomen. Thus, the abdomen seems to be a suitable thermal window for dissipating the surplus of heat when the risk of overheating is approaching. "Haemolymph shunting" from thorax to abdomen was first demonstrated by Heinrich (1970, 1971a, 1971b, 1976) in *Manduca sexta* and in bumblebees. In these, the blood flowing out of the thorax and the blood flowing into it (in the aorta) have to pass trough a narrow petiole and are thus in very close contact. This creates a counter-current heat exchange system helping to keep the heat in the thorax at low ambient temperature. Such a counter-current has also been identified in cuculiine moths by Heinrich (1987) who suggests that it helps maintaining the heat in the thorax when these moths warm up and fly at low ambient temperature (as low as 0 °C). When the insect risks overheating, the blood is pulsed alternately in and out of the thorax: the counter-current system is by-passed and hot blood passes to the abdomen where heat is lost through the uninsulated ventral surface. May (1976b, 1995) also found that dragonflies rely on heat transfer to the abdomen for thermoregulation, but pointed out that, in these insects, the increased rate of haemolymph circulation at high ambient temperature would be enough to explain the increased heat shunting. Flies from the *Eristalis* genus do not have a narrow waist, so a counter-current system seems unlikely. To

what extent eristaline flies might need to avoid overheating is open to discussion, as they are relatively small and are not particularly well insulated. However, some do live in very warm parts (e.g. Israel), and even in Britain ambient temperature can rise to above 30 °C.

Furthermore, apart from one unpublished study by Ellington (pers. comm.), to my knowledge no investigations of the endothermic capabilities of *E. tenax* and *E. pertinax* have been carried out to date. Ellington looked at the thorax temperature of the drone fly in free flight, and did not find any evidence of thermoregulation (pers. comm.).

1.3 Water balance

Water is the general solvent in an organism's body. Dehydration can have dramatic effects on the physiology of organisms. Enzymes may lose their function through changes in molecular shape, membranes may shrink and be disrupted, and the transporting fluid (for example blood) may become so viscous that it is difficult to circulate around the body. The problem for most terrestrial organisms is to conserve water, because their internal water activity (A_W) is equivalent to a relative humidity (RH) which is usually higher than that of the air (except in fully saturated air), and water is driven out of the body. For example, the water activity of insect haemolymph is equivalent to 99.5 - 99.8% RH (Wharton and Richards 1978), most of the time higher than ambient relative humidity. Water reserves in insects are replenished through feeding, drinking, water produced by the metabolism and, in some cases, active uptake of moisture from unsaturated air (Edney 1975, Willmer 1982a, Hadley 1994). Water is lost through evaporation, excretion, reproductive and defence fluids, etc. Evaporation comprises a respiratory component (through the spiracles), a cuticular component, and occasionally, evaporation from excreted fluids as a means of cooling down (Hadley 1994).

The arthropod cuticle is described by Locke (1975) and Hadley (1994). It is a non-cellular multilayered structure which is secreted from a single layer of epidermal cells just underneath it. Two major divisions are observed: the procuticle (bulk of cuticle, innermost) and the epicuticle (a

thin layer, uppermost). The outer part of the procuticle is sclerotized and is called the exocuticle; the inner part, the endocuticle, is soft. The cuticle is not uniform around the body; for example, a softer kind, the arthrodial membrane, is found at joints. Lipids are present in all the layers, but are found in greater amount in the epicuticle, making this layer the principal barrier to water loss. The composition of the epicuticular lipids is complex (Hadley 1994, Lockey 1988). The dominant lipids are the non-polar (thus, water repelling) lipids such as hydrocarbons. Straight chain hydrocarbons range in length from 20 to 37 carbon atoms. Longer chain-length hydrocarbons provide a better barrier to water loss as a result of their higher melting temperatures (Lockey 1988). Branched hydrocarbons and oxygenated hydrocarbon derivatives are also present in the total cuticular lipid fraction, as are aldehydes, ketones, and cholesterol, in smaller proportions. In addition, the epicuticle is sometimes covered by a layer of wax (wax molecules of varying complexity) which increases waterproofing (Hadley 1994). Straight chain hydrocarbons are found in almost every arthropod's cuticle but in varying proportions, their percentage of the total lipid fraction being apparently correlated with the organism's environment (Hadley 1994). For example, surface non-polar lipids occur in traces in tsetse flies (*Glossina*), for which water is readily available, but account for 98% of the lipid fraction in tenebrionid beetles, which live in xeric environments (Hadley 1994). Also, Hadley and Schultz (1987) showed that of three tiger beetle species, the one with the lowest water loss rate, *Cicindela obsoleta*, had also the greatest amount of hydrocarbons per surface area (0.077 mg $cm^{-2)}$ in its cuticle. *C. oregona*, which had the highest water loss, had the lowest hydrocarbon surface density (0.050 mg cm^{-2}). *C. obsoleta* inhabits dry grass land and is summer-active, whereas *C. oregona* is active in the spring and autumn, along water courses. However, Gibbs et al (1998) have shown that although hydrocarbon chain length increased when *Drosophila mogavensis* was acclimated to high temperature, lipid quantity did not change, and cuticular properties were not affected. They concluded that apparently adaptive changes in cuticular lipids do not necessarily improve cuticular resistance to water loses. Therefore, even when cuticular lipid composition is correlated with the arthropod's environment, the effect on the permeability of the cuticle should be experimentally investigated.

The organisation of the lipids in the epicuticle has been somewhat controversial. The polar lipid monolayer model was proposed by Beament (1958, 1961, 1964) to explain the sharp increase in cuticular water transpiration - and thus the change in the water proofing barrier - at a species specific "transition temperature" (below lethal temperature). Beament suggested that a monolayer of polar lipids located between the epicuticle and a layer of wax on the epicuticular surface was the principal barrier to water loss. The orientation of the polar lipids would change from 65° to the cuticle surface to 90° (becoming vertical) at the transition temperature, and permeability would increase. This model, which was once widely accepted by biologists, has now become controversial as non-polar lipids, not polar lipids, are now known to predominate in the cuticle. At present, there is no satisfactory explanation of the sudden increase in cuticular permeability at the transition temperature, and it might be that this phenomenon is a phase change (i. e. partial melting or conversion to a liquid crystalline state) of the non-polar lipids (Hadley 1994).

Water moves from internal tissues through the cuticle into the surrounding air, and the rate of this movement is usually expressed as permeability or resistance. Some areas of the cuticle are more permeable to water than others. For example, the thinner cuticle of the abdomen of the cricket *Acheta domesticus* is more permeable than its thicker thoracic cuticle (Hendricks and Hadley 1983). Also, the arthrodial membrane, which is found between metameric segments and at the joints of appendages, is not sclerotized and is more permeable than sclerotized cuticle (Hadley 1994).

Water loss is not uniform amongst arthropods, but depends on several factors:

- The resistance of the cuticle to water movement is correlated with the environment and is linked to the quantity of epicuticular lipids: high amounts of cuticular lipid are found in arthropods with low cuticular permeability (Hadley 1994). Temperature (ambient and surface) and humidity affect transpiration rates (Edney 1977, Willmer 1982a). The warmer and drier the environment, the more desiccating it is. Xeric insects, such as tenebrionid beetles, tend to have very low cuticle permeability and high cuticular lipid contents (Edney 1977). Also, as metabolism increases with temperature, so

do respiratory water losses (Hadley 1994). Quinlan and Hadley (1993) have shown that, in the grasshoppers *Romalea guttata* and *Taeniopoda eques*, metabolic rates increase by four to five fold when ambient temperature is raised from 15 to 30 °C; this is accompanied by a rise in cuticular and respiratory water losses.

- Cold is another environmental extreme. During overwintering, insects are likely to experience water loss with no possibility to replenish their reserves. Lundheim and Zachariasen (1993) have compared water losses of freeze tolerant (*Pytho depressus* larvae and *Upis ceramboides* adults) and freeze avoiding overwintering beetles (*Bolitophagus reticulatus*, *R. inquisitor* and *I. acuminatus*). They found that water losses of frozen beetles are lower than those of supercooled ones (because the haemolymph vapour pressure of supercooled insects is higher than that of frozen ones at the same temperature). However, freeze-avoiding species have lower cuticular transpiration rates, indicating that they compensate for their inability to tolerate freezing by being more efficient at saving water.

- Permeability and resistance to water loss also vary with age and stage of life cycle. For example, Pelletier (1995), studying the Colorado potato beetle, *Leptinotarsa decemlineata*, found that, when exposed to conditions of 30 °C and 5% RH for two to nine days, second instar larvae had a high mortality (70.2%) after two days; fourth instar larvae and adults survived a reduction of 60 and 48% of their body water over seven and nine days respectively; 48% of the pupae did not emerge as adults. He also found that the rate of water loss was lower in adults than in larvae, and that pupae had the lowest transpiration rates.

- The state of hydration is also important regarding transpiration rates. Several examples in the literature indicate that dehydrated arthropods have reduced water loss rates (e.g. Hadley and Quinlan 1993, Machin et al 1991) which can be achieved by the control of respiratory water losses and by the lowering of cuticular permeability (for example by increasing the amount of cuticular lipids).

Insects have several means to control their water losses. In general, insects are efficient osmoregulators and in extreme conditions some are also capable of withstanding a high level of osmotic stress. Arthropods' water loss tolerance ranges from 17 to 89% of their total water content (Hadley 1994). The control of water losses is achieved via both physiological and behavioural mechanisms. Physiological controls can be exerted on the excretory system, the respiratory system and the integument. The excretory system plays an important role in the maintenance of adequate concentration of salts and water in the body. The regulation of excretion is achieved via hormones both at the Malpighian tubules and the rectum, the major organs involved (Maddrell 1971, 1980, Bradley 1985, Hadley 1994). At their level water can be reabsorbed to produce more or less dry excreta. Water loss is also limited by the respiratory control of spiracles opening and cyclic CO_2 release, etc. Machin et al. (1991) supported the idea that ventilatory losses are lowered in dehydrated animals. However, Quinlan and Hadley (1993) shed doubts on the adaptive role of cyclic CO_2 release in two lubber grasshopper species. They pointed out that CO_2 cyclic release is only evident in quiescent insects (as was noticed by Machin et al 1991), dehydration disrupts the cycle, and respiratory losses are such small percentages of total water losses that there is not much scope to lower them in resting insects. Cuticular permeability can also be altered. A surface layer of wax can lower evaporation. Water losses can be reduced by increasing the cuticular lipid contents, in response to becoming acclimated to a more desiccating environment (Gibbs 1998). Hadley (1977) demonstrated that winter active tenebrionid beetles (*Eleodes armata*) have lower amounts of hydrocarbons and higher proportions of long chain lipids than summer active ones, which have a higher risk of desiccation. Moreover, keeping winter beetles for five weeks at 35 °C resulted in an increase of their cuticular lipid contents. In addition, it has been suggested by Treherne and Willmer (1975) that integumental water loss could be under hormonal control in the cockroach, *Periplaneta*. This issue is still controversial however, and requires more experimentation (Hadley 1994).

Metabolic water production is also an important factor in the maintenance of water balance in active insects. Louw and Hadley (1985) showed that metabolic water production almost compensates for water

losses in hovering honeybees. They estimated that a hovering honeybee loses 79.7 mg g^{-1} h^{-1} but that metabolic water is produced at the rate of 74.4 mg g^{-1} h^{-1}. The same seems true for flying carpenter bees (below ambient temperature of 27 °C) (Nicolson and Louw 1982).

Behavioural controls of water balance also directly affect the body's thermal balance. An insect retreating to a cool, humid place to reduce its loss of water will also cause its body temperature to decrease. On the other hand, basking to raise body temperature will inevitably increase water losses. Pelletier (1994) showed that, in the Colorado potato beetle, the rate of water loss rises with increasing ambient temperature and with decreasing RH. An active insect, with a high body temperature and metabolic rate, will open its spiracles more often to obtain oxygen and will lose more water through respiration, as was also shown by Machin et al (1991) and Quinlan and Hadley (1993). Therefore, insects are often forced to compromise between being active and conserving water (Willmer 1982a). Evaporative cooling is usually precluded for insects because of their small size and small water reserve. However, there are exceptions: if body temperature gets dangerously high, evaporation of water, for example from salivary droplets in honeybees, can be used to cool down (Edney 1977, Heinrich 1980a, b). Toolson (1987) and Hadley et al (1989) demonstrated the role of evaporative cooling in the desert cicada *Diceroprocta apache*. At high temperature (45.5 °C) and 0% RH, body temperature is maintained at 39.5 °C. If prevented from evaporating water by being placed in 100% RH, the cicada's body temperature increases to ambient temperature.

As with thermoregulation, water balance is also affected by the individual's size and shape. A large surface area to volume ratio means relatively more surface for transpiration; but being small allows the exploitation of microclimates. Thus, although small arthropods lose water relatively faster than large ones, they can use microenvironments provided by the vegetation or cracks in rocks, for example, to limit their losses. Retreating in a suitably humid microhabitat could well be one of the means female drone flies use to maintain their water balance while overwintering. Aggregation is an efficient means to increase effective size and limit water evaporation (Willmer 1982a). In addition, the presence of hair/fur can lower water losses in the same way as it decreases heat

losses, by trapping a layer of air within which temperature/humidity gradients become established.

During the active period (spring and summer), *E. tenax* and *E. pertinax* gain water through feeding (nectar) and drinking (Gilbert 1985b) and through metabolic water production. Water contents are probably regulated by behavioural and physiological means. The situation is different for overwintering flies; there is a need to be highly economical both with the energy invested for physiological processes (including those involved in water regulation) and with water. Although the drone fly has been reported to fly in winter months (Siuda 1963, Kato 1943, Hastings 1988, Gilbert pers. comm.) when air temperature and solar radiation are adequate, the frequency of such excursions is not known. It would be expected that, unless food sources are available (as in Kew gardens, Hastings 1988), these flights should be limited in order to conserve energy reserves. Thus, it is likely that *E. tenax* relies on its water reserves for considerable periods. *E. tenax* overwinters in crevices in caves and between stones in old buildings; such places are likely to provide a suitably humid microenvironment to limit water losses. A study of cave environments by Howarth (1980) confirmed the high humidity of such sites. Low activity also helps in reducing water losses. Physiological strategies could also be used. For example, the cuticle permeability could be decreased, but this is likely to require investments (such as lipids incorporated in the cuticle, hormones, etc.) that might need to be limited in order to maximise energy reserves. In addition, female *E. tenax* might increase their fat reserves prior to overwintering, to ensure that enough energy is available for the winter. Fat bodies are relatively free of water; hence, individuals with extensive fat deposits usually have low water reserves (Hadley 1994). If female drone flies increase their fat reserves prior to overwintering, they might have to compromise on their water reserves. Trades off between energy and water reserves are likely to arise for overwintering *E. tenax*. During the several months spent overwintering, *E. tenax* will certainly be subject to water loss. There has been no study to date on the ability of *E. tenax* to withstand dehydration. Whether the selection of a suitable microenvironment is enough to limit water losses or physiological regulation of these losses is necessary will be

investigated in this project. In summer, although activity and a higher temperature mean increased water losses, Scottish *E. tenax* and *E. pertinax* should not experience problems to replenish their reserves.

1.4 Influence of climatic conditions on the activity of insects

From the account given above about the problems faced by insects trying to regulate their body temperature and to conserve water, it can be expected that climatic conditions will greatly influence the activity of insects. Foraging, for example, requires that the animal gets warm enough to be active; but the insect must also control its water losses incurred through the higher body temperature and the respiratory demands of activity. In addition, the energy rewards must be large enough to over-compensate the costs incurred. Thus, it is likely that foraging time will be determined by the complex interaction of these factors. Several studies demonstrate the influence of climatic conditions on insects' activities. Willmer (1982b) showed that the abundance of flies visiting water-lily leaves, a resting site, followed a diurnal pattern related to the microclimatic conditions at the leaf surface. The visit timing differed between species, and was related to the size and reflectance of the insect: larger less reflective forms occurred at night, dawn, and dusk; smaller highly reflective ones peaked at the hottest part of the day. The influence of the size (mass) appeared greater than that of reflectance. On a dull day, the distribution was more unimodal with the peak at the warmest time of the day. This demonstrates the influence of the weather. The leaves were most used when the humidity difference between ambient air and moister air just above the leaves was greater, suggesting an avoidance of water losses. These findings suggest that activity is influence by hygrothermal conditions, the most important being an appropriate body temperature and the avoidance of heat stress. Willmer (1983) also studied the timing of foraging, an activity where hygrothermal considerations must be balanced with food availability. Again, size and reflectance were shown to affect activity in relation to radiation. As radiation, thermal costs, and nectar rewards (sugar amounts) are mutually dependent parameters, it is very

difficult to analyse the influence of any one of them on the activity pattern. For example, both insect activity and nectar concentration increase with solar radiation. But the increase in insects visiting flowers with the increase in nectar concentration is probably incidental, and is a result of thermal requirements, insect number increasing with solar radiation; in the conditions of the study (hot and dry), it is unlikely that insects would benefit by foraging on more concentrated nectar which is more difficult to obtain. Interestingly, *Bombus*, an endothermic bumblebee, was shown to forage mainly early in the day and, to a lesser extent, in the evenings. Its endothermic capabilities have freed it from solar radiation and temperature dependence; thus, it can forage when nectar is most abundant, and when the risk of overheating is reduced. A study of the bee *Chalicodoma sicula* by Willmer (1986) showed that this bee, which lives and nests in arid parts of Israel, adjusts its foraging pattern more to control its water balance than to maximise its energy input. The volume of water collected was highly correlated with the ambient temperature, and smaller flies had to make more trips. This monolectic bee can, to a certain extent, select patches offering hygrothermally suitable conditions, but cannot select the amount of water and calories independently, as can some sphecids which forage on various different species of flowers (Willmer 1985). It seems that in the case of *Chalicodoma* the strategy is to forage for the correct amount of water assuming that then adequate energy will be gained, as water is the most limiting constraint. As bee and flower (*Lotus creticus*) have probably co-evolved, this is presumed to be a suitable strategy. When *Chalicodoma* is forced to forage on another legume producing more concentrated nectar it adjusts its water intake by visiting labiate flowers offering dilute nectar. Thus, the influence of climatic conditions on insects' activities depends on the interaction of numerous factors such as the morphology and physical properties of the insect, its natural history, and the constraints it is subjected to (water conservation, temperature regulation, etc.).

The activity patterns of hoverflies have been studied by various people. Willmer (1983) classified flies from the genus *Eristalis* as large (above 75 mg) and with very low reflectance (below 3%). As expected, their activity is not correlated with radiation; they forage throughout the day but with peaks of abundance at dawn and dusk, on a partially shaded

site; and in the morning, avoiding the hottest period of the day, at a site more exposed to the sun. Gilbert (1985b) confirmed that *E. tenax*, being a large species, is one of the first to become active in the morning. This is probably an advantage as both pollen and nectar are usually produced in the morning in Northern temperate flowers (Corbet 1978, Corbet *et al.* 1979, Willmer 1983). The possible endothermic capabilities of the drone fly could also free it, to a certain extent, from thermal climatic constraints. Gilbert (1985b) reported that the lowest temperature at which *E. tenax* was observed was 10 °C and the lowest temperature at which more than 50% of the flies were active was 11 °C. Kato (1943) observed flying *E. tenax* in November and December, in Japan, at air temperatures as low as 4.5 °C. He found that, in the laboratory, certain ambient temperatures are required for *E. tenax* to become active: cleaning started at 8 °C, crawling at 12 °C, and normal activity at 15 °C. In the field, it seems that the fly is able to use solar radiation to warm up (by basking), and thus, can fly at an ambient temperature lower that this minimum, provided that the sun is shining (Kato 1943). Kikuchi (1965) observed that on oxeye daisy, *Chrysanthemum leucanthenum*, the appearance of *E. tenax* was induced mainly by temperature: it appeared at 16-20 °C and maximum numbers were observed at 25-28 °C. Above solar radiant heat of 28 °C *E. tenax* was not active and migrated behind the petals of the flower. Gilbert (1985b) observed that, on average, in the drone fly, feeding took 79.3% of the time, resting 13.1%, and flying 0.6%. In general, female hoverflies spend more time feeding than males. He found that *E. tenax* was significantly more likely to be seen in the sun than in the shade, probably reflecting the use of solar radiation for temperature regulation. In the early hours, pollen feeding dominates; nectar feeding increases to reach a peak around midday. This does not seem in accordance with Willmer's (1983) findings, but could only reflect differences in ambient temperatures which were lower in Gilbert's study (up to 25 °C, compared to up to 34 °C in Willmer's study).

1.5 Aims of this thesis

This study involves both behavioural and physiological studies of *E. tenax* and *E. pertinax*.

E. tenax provides a very good opportunity to study differences in physiological performances, such as thermoregulation and maintenance of water balance, in two generations (summer and winter). *E. pertinax* is a closely related species which shares various ecological aspects with *E. tenax*, for example feeding sources, but also presents some differences (for example it does not overwinter as an adult, and males seem territorial whereas male *E. tenax* do not seem to be so in Britain). Thus, the behaviours and the physiological abilities of two closely related species can be compared.

As size is such an important factor for the physiology of insects, Chapter 2 concentrates on the relationship between linear dimensions and mass in the two species. The consequences of using only one size factor are discussed. These findings are used in most of the following chapters where the effect of size on the biology of these eristalines is investigated.

Chapter 3 analyses the water balance limits of the two species using laboratory studies. The two generations of *E. tenax* and the two species are compared for their ability to control water losses.

Chapter 4 aims at understanding the purely physical aspects of the thermal biology, i.e. the passive rates of heat exchange, of these flies. The effects of size and evaporative water loss on the passive rates of heat exchange are investigated.

Chapter 5 addresses the endothermic and thermoregulatory capabilities of *E. tenax* and *E. pertinax* in relation to the flies' size, ambient and body temperature. The suitability of the "grab and stab" method to estimate the body temperature of free ranging flies is assessed by comparing field data with laboratory data, where body temperature is continuously recorded. Voluntary flight temperature and stable flight temperature are measured in "free" flying flies (attached to a fine thermocouple but not supported by the thermocouple).

The results of a preliminary study on overwintering in female *E. tenax* are shown in Chapter 6. The characteristics of the overwintering sites, the behaviour of the flies during this period and the factors affecting the start and end of overwintering are discussed.

Chapter 7 investigates various aspects of the behavioural ecology of these species such as foraging patterns, choice of flower, activity patterns, territoriality, and mating behaviour. The influence of environmental factors, like temperature, humidity light, food rewards and time of day on these activities are investigated.

A general conclusion, drawing all the threads together, constitutes Chapter 8.

Chapter 2 - Morphometrics

2.1 Introduction

Size is a very important factor for living organisms, as it determines their way of life: locomotion, habitat choice, feeding, susceptibility to environmental changes, etc. Physiological processes, such as thermoregulation, are very much influenced by size (for example May 1976a and b, Bartholomew and Heinrich 1977, Morgan and Heinrich 1987, Willmer 1982, Stone and Willmer 1989a) as will be seen in this thesis. For example, large animals are less affected by temperature changes in their environment because they have a small surface area to volume ratio, but their size prevents them from occupying microhabitats that provide a stable microclimate.

Mass is quite often used as a measure of size. It is easy to measure, even in the field (especially in insects), and it does not require killing the animal. However, and in particular in insects, mass is not always the best size parameter when investigating the influence of size on a physiological process. For example, when the rate of endothermic warm-up in insects is considered, the size of the thorax (mass or width) might be of greater influence than body mass because the muscles used for warming up are contained in the thorax. The same is true for cooling rates in insects (Bartholomew and Epting 1975). Moreover, individuals can vary widely in mass depending on their feeding state or gravidity.

In addition to size, shape is also an important parameter: a flattened object has a larger surface area to volume ratio than a rounded one of the same mass and will be more affected by changes in its environment.

In this thesis, three size factors are going to be relevant in physiological studies: mass (m), thoracic width (Th_w, assumed to be correlated with other linear dimensions), and thoracic mass (m_{th}, assumed

to be correlated with thoracic muscle mass). The relevant size factors depend on the physiological process being studied, and some physiological processes might be influenced by more than one size factor. Where linear dimensions are relevant, thoracic width will be an appropriate factor; where muscle power is important, thoracic width or thoracic mass will be adequate size factors. For example, stable flight temperature might be related to both mass and thoracic size, because it depends on the power of the flight muscles and on the weight which has to be supported. Mass was recorded in all the studies, thoracic width was measured in laboratory studies, and thoracic mass was never determined (except for this chapter), because flies were kept intact for future reference. At the beginning of this project, it was assumed that all three size factors would be correlated with each other, and that constructing calibration curves would allow the estimation of the other two factors when one of them is known. This idea was particularly appealing because it is often difficult to measure several size factors, particularly in the field. Mass is usually the easiest parameter to measure. In addition, the determination of thoracic mass involves killing the insect; it seemed a waste to kill every fly sampled just for this purpose.

Females and males of the same species do not necessarily have the same relationship between their linear dimensions and their mass, or their body part masses. It is therefore worth investigating these relationships before considering the influence of size on physiological processes, as a sex difference might in fact reflect sex dimorphism rather than a physiological difference.

Here, the relationship between body mass, thorax mass and thorax width will be investigated for the two sexes of *E. tenax* and *E. pertinax*.

2.2 Materials and methods

Freshly killed flies were weighed (electronic balance, Sartorius Handy H160, Sartorius Ltd., UK). Their thorax width was measured using a binocular microscope with an eye-piece graticule. The flies were then dissected and their thorax (without the wings) was weighed.

All data sets were tested for normality and, if necessary, transformed before using any parametric test. All the tests were carried out using "Minitab" version 8.2 on an Apple Macintosh.

Regression was used to analyse the effects of continuous variables on one another. The equation of the best fitted line is given either in the text or in the figure legend; n, p and R^2 values are given in the text.

The general linear model was employed (because of unequal sample sizes) to investigate the effect of a number of variables (non-continuous and continuous, in which case a covariance analysis was done) on another. The results are presented in tables.

2.3 Results

2.3.1 *E. tenax*

Table 2.1 shows the mean values and ranges for mass, thoracic width and thoracic mass for the two sexes of each species.

Table 2.1 Mass, thoracic width and thoracic mass in *E. tenax* and *E. pertinax*

	Mean mass [range] (mg)	Mean thoracic width [range] (mm)	Mean thoracic mass [range] (mg)
Fem. *E. tenax* (n=23)	144.3 [75.0-211.0]	3.99 [3.55-4.25]	49.7 [30.0-63.0]
Male *E. tenax* (n=23)	108.6 [63.0-176.0]	3.83 [3.15-4.15]	45.2 [29.0-59.0]
Fem. *E. pertinax* (n=22)	88.8 [26.0-148.0]	3.49 [2.85-2.90]	33.9 [11.0-49.0]
Male *E. pertinax* (n=20)	74.6 [30.0-117.0]	3.52 [2.80-3.85]	34.8 [14.0-47.0]

The relationships between mass, thorax width and thorax mass are better described using logarithms.

A covariance analysis (Table 2.2) looking at the effect of the logarithm of thoracic width, sex and the interaction between the logarithm of thoracic width and sex on the logarithm of mass shows that there is a strong positive relationship between the logarithm of mass and the logarithm of thoracic width, and that the relationship differs between males and females (significant interaction between the logarithm of thoracic width and sex). Also, once thoracic width has been controlled for, females are heavier than males. Figure 2.1a shows the relationship between the logarithm of mass and the logarithm of thoracic width for females and males (females: n=23, R^2=0.62, $p<0.001$; males: n=23, R^2=0.30, p=0.007). As thoracic width increases, the mass of females increases faster than the mass of males. As a result, large females are heavier than large males, whereas for flies with thoracic widths in the middle of the range, both sexes are about the same mass.

Table 2.2 Covariance analysis on logm for $logTh_w$, sex and the interaction between $logTh_w$ and sex in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
$LogTh_w$	1	0.408	0.329	0.329	39.80	<0.001
Sex	1	0.037	0.046	0.046	5.54	0.023
$LogTh_w$*Sex	1	0.050	0.050	0.050	6.00	0.019
Error	42	0.347	0.347	0.008		
Total	45	0.842				

Fitted means for logm:

	Mean (mg)	Stdev (mg)
Male	2.042	0.020
Female	2.096	0.020

Similarly, the covariance analysis (Table 2.3) on the logarithm of thoracic mass for the logarithm of thoracic width, sex and the interaction between the logarithm of thoracic width and sex reveals a positive relationship between the logarithm of thoracic mass and the logarithm of thoracic width which is different in each sex. Also, females tend to have a heavier thorax than males. Figure 2.1b shows the relationship between the logarithm of thoracic mass and the logarithm of thoracic width for females and males (females: n=23, R^2=0.80, p<0.001; males: n=23, R^2=0.77 p<0.001). Thoracic mass in females increases faster with thoracic width than in males. Females have heavier thoraxes than males, but that essentially applies to the upper end of the thoracic width range; at the lower end females tend to have lighter thoraxes .

Table 2.3 Covariance analysis on $logm_{th}$ for $logTh_w$, sex and the interaction between $logTh_w$ and sex in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
$LogTh_w$	1	0.025	0.023	0.023	147.09	<0.001
Sex	1	0.0009	0.0015	0.0015	9.40	0.004
$LogTh_w$*Sex	1	0.0014	0.0014	0.0014	8.70	0.005
Error	42	0.0066	0.0066	0.0002		
Total	45	0.033				

Fitted means for $logm_{th}$:

	Mean (mg)	Stdev (mg)
Male	0.589	0.0027
Female	0.597	0.0027

The covariance analysis (Table 2.4) on the logarithm of thoracic mass for the logarithm of mass, sex and the interaction between the logarithm of mass and sex does not, however, show any difference between the sexes in the relationship between the logarithm of thoracic mass and the logarithm of mass and, once the logarithm of mass has been controlled for, neither sex has a heavier thorax than the other (even when the interaction between the logarithm of mass and sex is omitted from the analysis). Figure 2.1c shows the relationship between the logarithm of thoracic mass and the logarithm of mass for females and males (females: n= 23, R^2=0.65, p<0.001; males: n=23, R^2=0.38, p=0.002). Thoracic mass increases with mass at a similar rate in both sexes, and for a similar mass, males and females have a similar thoracic mass.

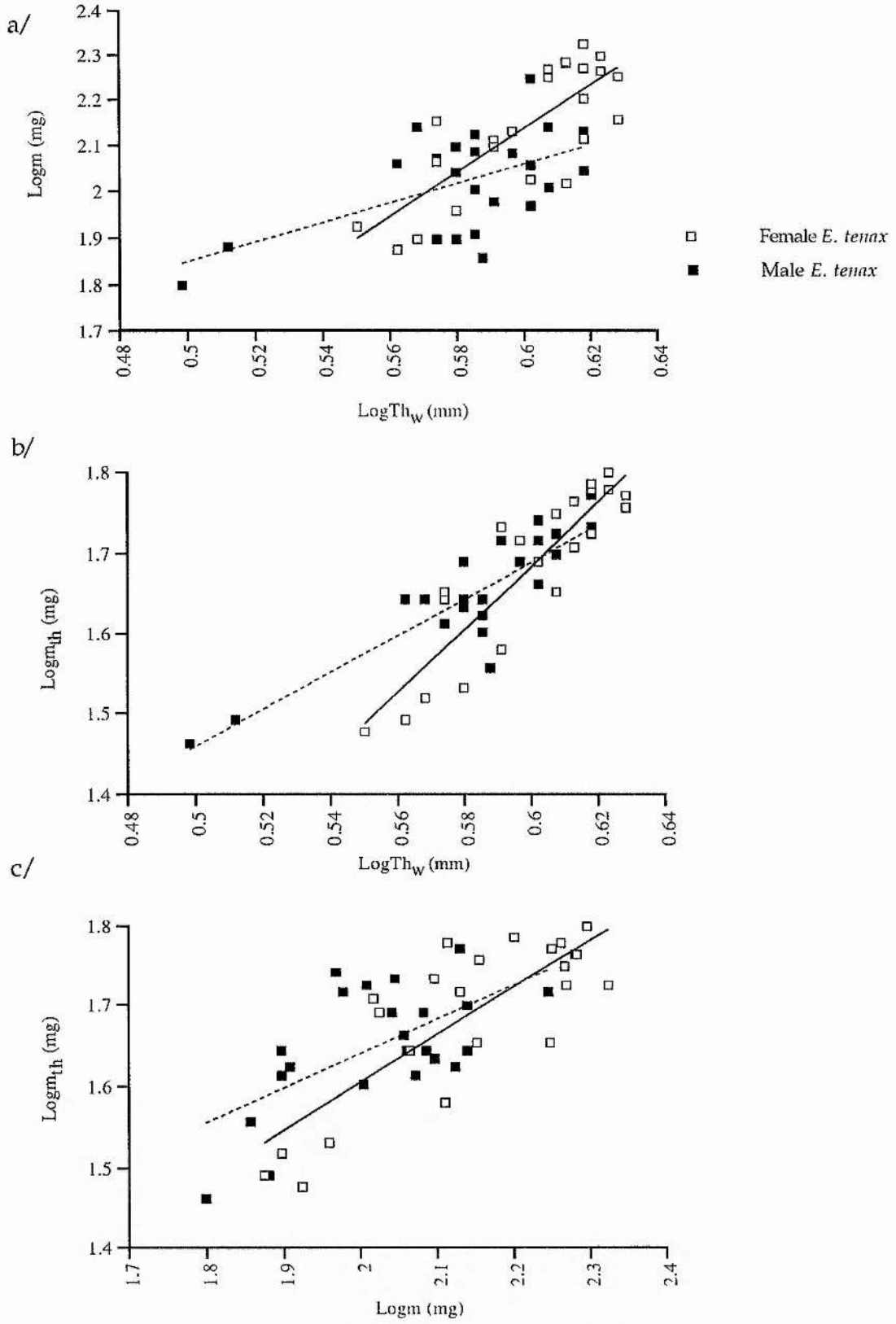


Fig. 2.1 Relationships between mass, thoracic mass and thoracic width in *E. tenax*

a/ Logm vs logTh$_w$. Females: $y = 4.76x - 0.72$, $r^2 = 0.62$; males: $y = 2.10x + 0.80$, $r^2 = 0.30$
b/ Logm$_{th}$ vs logTh$_w$. Females: $y = 3.94x - 0.68$, $r^2 = 0.80$; males: $y = 2.31x + 0.31$, $r^2 = 0.77$
c/ Logm$_{th}$ vs logm. Females: $y = 0.59x + 0.43$, $r^2 = 0.65$; males: $y = 0.42x + 0.79$, $r^2 = 0.38$

Table 2.4 Covariance analysis on $logm_{th}$ for logm, sex and the interaction between logm and sex in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Logm	1	0.199	0.166	0.166	44.84	<0.001
Sex	1	0.005	0.005	0.005	1.37	0.249
Logm*Sex	1	0.004	0.004	0.004	1.20	0.280
Error	42	0.156	0.156	0.004		
Total	45	0.364				

Fitted means for $logm_{th}$:

	Mean (mg)	Stdev (mg)
Male	1.674	0.015
Female	1.652	0.014

As explained above, *E. tenax* females have larger gradients than males for the regression lines of the logarithm of mass on the logarithm of thoracic width and of the logarithm of thoracic mass on the logarithm of thoracic width. This means that mass and thoracic mass increase faster with thoracic width in females than in males. Moreover, because the relationship between the variables is logarithmic, for large flies, thoracic width does not increase as fast with mass or thoracic mass as it does in smaller flies: there is a plateau, where mass increases whereas thoracic mass does not change much. This plateau is more marked in females (reflected in the greater gradients), although it also exists for males.

2.3.2 *E. pertinax*

Again, the relationships between mass, thoracic width and thoracic mass are better described using logarithms. No interaction between either of these variables and sex was found: males and females have similar relationships between thoracic width, mass and thoracic mass (the gradients of the regression lines of the logarithm of mass on the logarithm of thoracic width, the logarithm of thoracic mass on the logarithm of thoracic width and the logarithm of thoracic mass on the logarithm of mass are similar in both sexes). However, when interactions are omitted from the analyses, sex differences appear.

A covariance analysis on the logarithm of mass for the logarithm of thoracic width and sex (Table 2.5) shows that there is a positive relationship between the logarithm of mass and the logarithm of thoracic width and that females are heavier than males (once thoracic width has been controlled for). Figure 2.2a shows the relationship between the logarithm of mass and the logarithm of thoracic width for both females and males (females: n=22, R^2=0.60, p<0.001; males: n=20, R^2=0.77, p<0.001). So, as thoracic width increases mass increases. Females' mass changes with thoracic width in the same way (same gradient) as males' mass (the interaction between the logarithm of thoracic width and sex is not significant: the regression lines of the logarithm of mass on the logarithm of thoracic width are parallel). However, the regression line for females lies above the one for males: females are heavier than males with a similar thoracic width, and the difference in mass is constant all throughout the range.

Table 2.5 Covariance analysis on logm for $logTh_w$ and sex in E. *pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
$LogTh_w$	1	0.659	0.684	0.684	76.50	<0.001
Sex	1	0.071	0.071	0.071	7.90	0.008
Error	39	0.349	0.349	0.009		
Total	41	1.08				

Fitted means for logm:

	Mean (mg)	Stdev (mg)
Male	1.844	0.021
Female	1.926	0.020

However, the covariance analysis on the logarithm of thoracic mass for the logarithm of thoracic width and sex does not show any difference between the sexes (Table 2.6). Figure 2.2b shows the regression of the logarithm of thoracic mass on the logarithm of thoracic width for both females and males (females: n=22, R^2=0.80, p<0.001; males: n=20, R^2=0.89, p<0.001). Thoracic mass increases with thoracic width at the same rate for both sexes. Moreover, males and females with a similar thoracic width have a similar thoracic mass.

Table 2.6 Covariance analysis on $logm_{th}$ for $logTh_w$ and sex in E. *pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
$LogTh_w$	1	0.666	0.662	0.662	197.43	<0.001
Sex	1	0.00005	0.00005	0.00005	0.02	0.903
Error	39	0.131	0.131	0.003		
Total	41	0.797				

A covariance analysis on the effect of the logarithm of mass and sex on the logarithm of thoracic mass was carried out (Table 2.7). It shows that the logarithm of thoracic mass and the logarithm of mass are positively correlated and that, once the logarithm of mass is controlled for, males have heavier thoraxes than females. Figure 2.2c shows the relationship between the logarithm of thoracic mass and the logarithm of mass for females and males (females; n=22, R^2=0.83, p<0.001; males: n=20, R^2=0.83, p<0.001). The two lines are parallel, with the one for males being above the one for females. Thus, thoracic mass increases with body mass at the same rates in both sexes, and males have heavier thoraxes than females of similar mass.

Table 2.7 Covariance analysis on $logm_{th}$ for logm and sex in E. *pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Logm	1	0.608	0.655	0.655	185.27	<0.001
Sex	1	0.050	0.050	0.050	14.18	0.001
Error	39	0.138	0.138	0.004		
Total	41	0.797				

Fitted means for $logm_{th}$:

	Mean (mg)	Stdev (mg)
Male	1.554	0.013
Female	1.483	0.013

Figure 2.3a, b & c shows the relationships between the logarithms of thoracic width, mass and thoracic mass for both sexes of the two species.

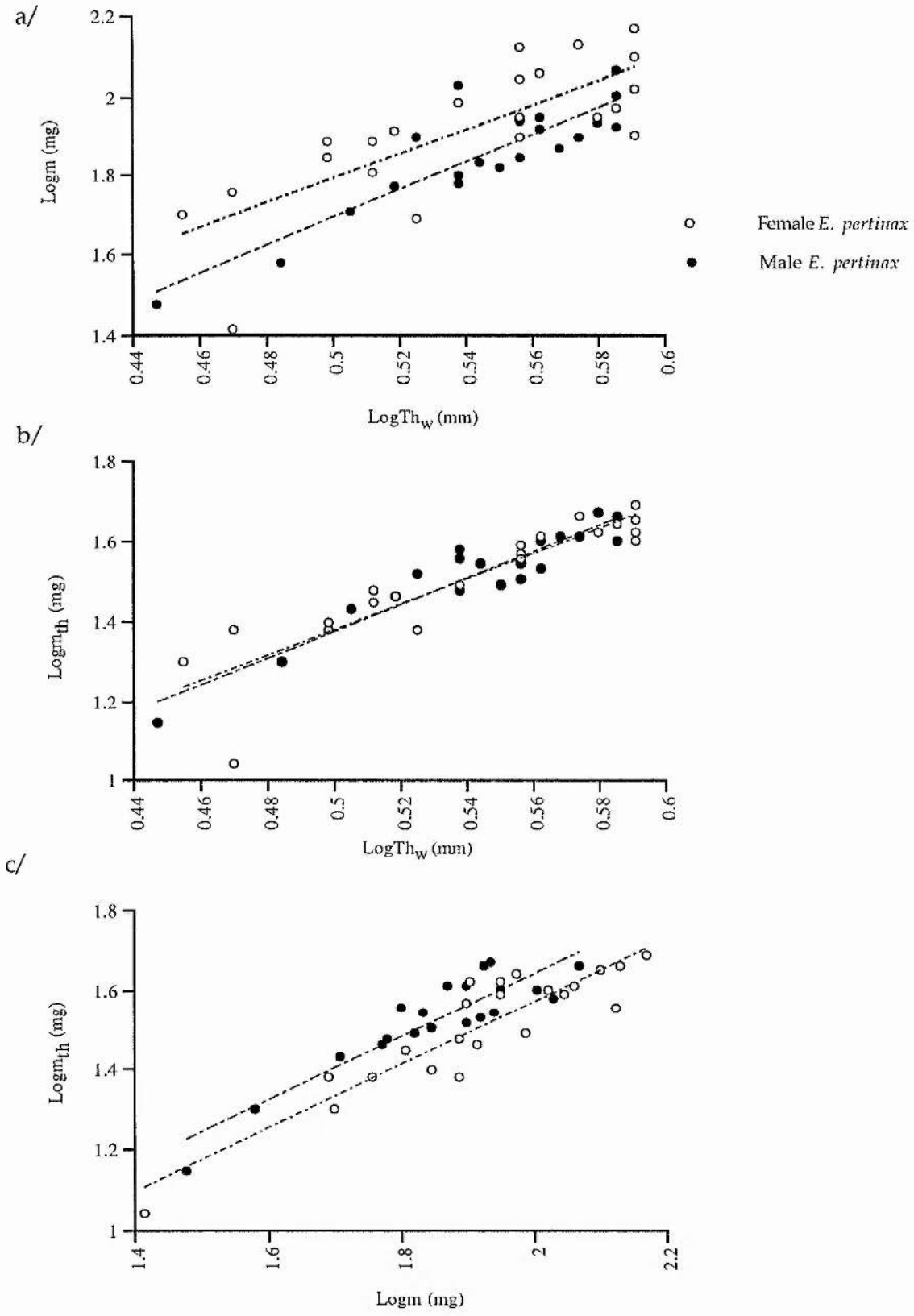


Fig. 2.2 Relationships between mass, thoracic mass and thoracic width in *E. pertinax*

a/ Logm vs logTh$_w$. Females: y = 3.12x + 0.24, r^2 = 0.60; males: y = 3.51x -0.06, r^2 = 0.77
b/ Logm$_{th}$ vs logTh$_w$. Females: y = 3.15x - 0.20, r^2 = 0.80; males: y = 3.31x - 0.28, r^2 = 0.89
c/ Logm$_{th}$ vs logm. Females: y = 0.80x - 0.02, r^2 = 0.83; males: y = 0.80x + 0.05, r^2 = 0.83

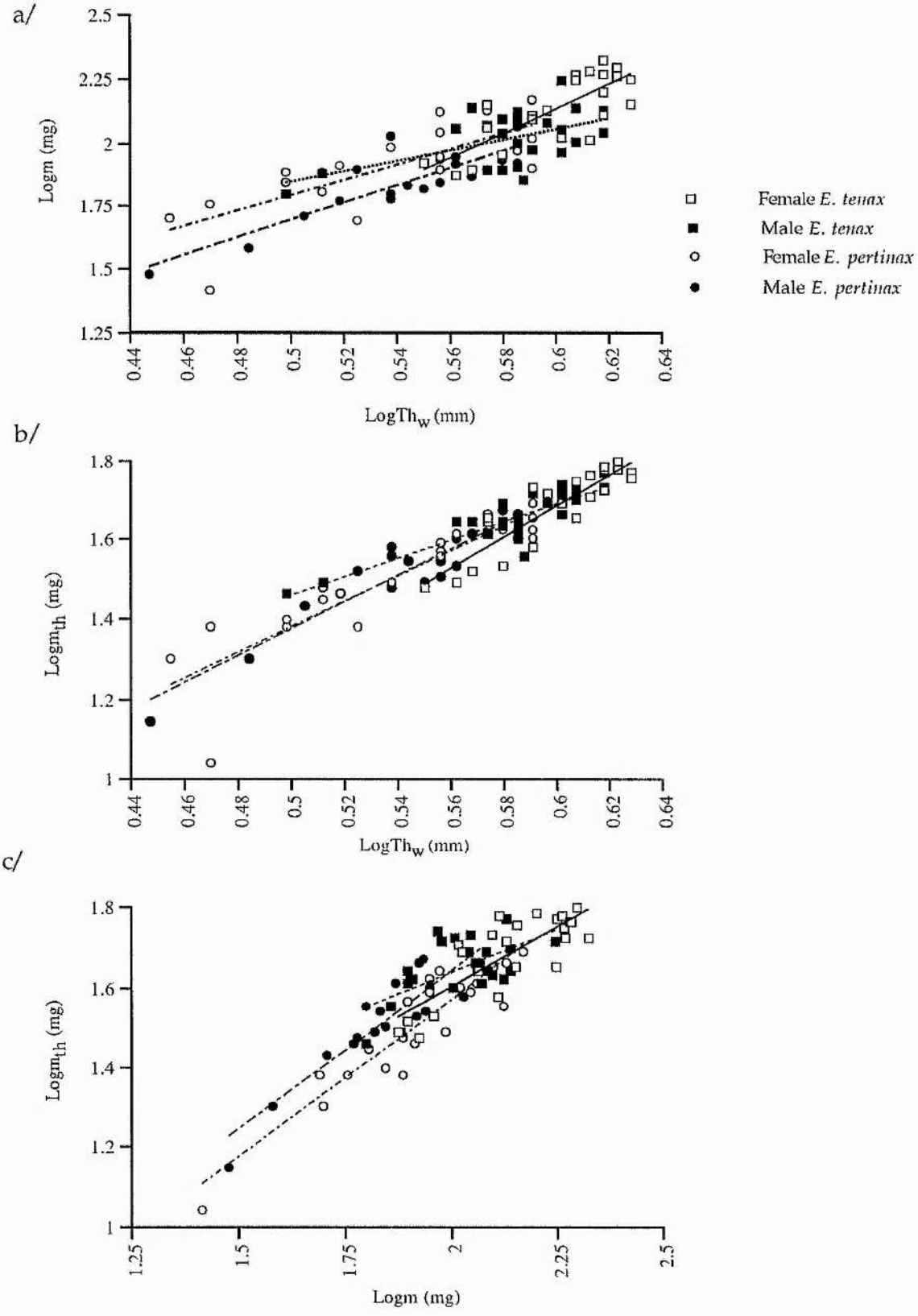


Fig. 2.3 Relationships between mass, thoracic mass and thoracic width in *E. tenax* and *E. pertinax* (see equations for the regressions in Fig. 2.1 and Fig. 2.2)

a/ Logm vs $logTh_w$
b/ $Logm_{th}$ vs $logTh_w$
c/ $Logm_{th}$ vs logm

This figure is intended to show the size range and any gross difference between the linear dimensions and body mass and thoracic mass of the two species. No statistical analysis was carried out to investigate species difference because of the existing sexual differences within the species. The sexes cannot be lumped together (within a species) for such an analysis. If the sexes are taken into account, it is not sufficient to include sex as a factor in the analysis because it cannot be assumed that females (or males) of both species have the same relationship between the variables under consideration. It would be necessary to separate female *E. tenax*, female *E. pertinax*, male *E. tenax* and male *E. pertinax*, but this leads to colinearity problems in the covariance analysis. So, the difference in the relationships between the variables (difference in gradient of the regression lines) in the two species was not investigated. Proper care will be taken for each particular analysis in this thesis where such a problem could arise. However, looking at Figure 2.3, the three size parameters seem similar where the two species overlap in size. For example, for a particular thoracic width, *E. tenax* does not seem to be heavier than *E. pertinax*. Moreover, Figure 2.3 shows that *E. tenax* (especially females) is overall a bigger fly than *E. pertinax* .

2.4 Discussion

2.4.1 *E. tenax*

The interaction between thoracic width and sex is significant in the analyses of the effect of these factors on mass and thoracic mass, but there is no difference between males and females for the relationship between mass and thoracic mass. Thus, males and females differ in their relationships between linear dimensions and mass and thoracic mass: they are of a different shape. For a similar thoracic width, females tend to be heavier and to have a heavier thorax than males (the thorax of females has a bigger volume for the same width compared with the thorax of males). Although gravidity in females might also contribute to some extent to the mass difference, the shape difference is certainly more important (as there

is no difference between the sexes in the relationship between mass and thoracic mass). So there is a dimorphism between the two sexes of *E. tenax*.

2.4.2 *E. pertinax*

Males and females have the same relationship between their linear dimensions and their mass and thoracic mass. This was demonstrated by the absence of any significant interaction between sex and the variables investigated and the absence of a sex difference in the relationship between thoracic mass and thoracic width (for similar thoracic widths, males and females have similar thoracic masses). Therefore, males and females are of the same shape. However, females tend to be heavier than males. For a similar thoracic width, females are heavier than males and for a similar mass they have a lighter thorax. Thus, it is clear that the difference comes mainly from the abdomen, probably reflecting food contents and gravidity.

2.4.3 Implications

Before the morphometrics of these species were investigated, it was thought that all three size parameters would be well correlated, and that knowing one of them would allow the accurate determination of the others. It is now clear that this is not the case. First, there is considerable variation in mass amongst the flies because of different feeding state and hydration (as will be seen later, considerable mass loss occurs through loss of water, and some flies were found to lose as much as 42% of their mass during thermal balance experiments) and of gravidity in females. Thus, mass does not always reflect size and muscle power accurately. As the relationships between the three variables are logarithmic, mass factors change more rapidly than thoracic width in large flies: flies of similar thoracic width can have very different masses.

For both *E. pertinax* and *E. tenax*, when one size factor only is likely to influence the variable investigated and it is used in the analysis, controlling for it and for sex will at the same time control for any sex effect linked to this size factor. For example, controlling for mass will eliminate the sex difference linked to one sex being heavier than the other. However, the situation is more complex when two size factors are involved because of the sex differences in the relationships between the size factors.

Investigation of stable flight temperature is such a situation: both mass and flight muscle power are likely to have an effect on this variable. We have seen that for both *E. tenax* and *E. pertinax*, females are heavier than males with the same thoracic width. For *E. pertinax*, the difference between the two sexes is only related to mass and not to shape. Thus, controlling for mass and sex will resolve the matter and eliminate the sex difference linked to size. For *E. tenax*, the situation is more complex because females and males are of different shape. If both mass and thoracic width can be used in the analysis, when they and sex are controlled for, the sex difference linked to size will be eliminated. If sex remains a significant predictor of stable flight temperature, the sex effect is a "real" one, i. e. it is not linked to size difference. Moreover in *E. tenax*, there might not be any clear sex effect or a sex effect might only appear at the extreme of the size range. For example, heavy females will have considerably smaller thoracic widths than males of same mass, but flies of medium mass will not differ much in their thoracic width (see Chapter 5, "grab and stab").

It is therefore very important, when size is thought to influence a variable that several size factors should be considered and controlled for together with sex to avoid interpreting a sex difference linked to size as a "real' sex difference. However, it is often the case, especially where field work is involved, that only one size factor is available. In such a situation, when sex is a significant predictor of the variable studied, and there is good reason to believe that another size factor could also influence this variable, care should be taken in the interpretation of the analysis in *E. tenax*: a sex difference could just reflect a size difference between the sexes.

Overall, when ignoring the shape of the relationships between the three size parameters, *E. tenax* and *E. pertinax* are not morphologically dissimilar, i.e. flies of similar linear dimensions have a similar mass. Thus, in a physiological process, a species difference should not arise from a morphological difference (like, for example, one species being heavier than another at similar thoracic widths). Problems with analysis and interpretation will arise when a sex difference is found in one or both species because of the difficulties with statistical analyses explained above. Finally, it is clear from these analyses that *E. tenax* is a bigger fly than *E. pertinax* and might have different physiological abilities simply because of its larger size.

This chapter has demonstrated the importance of investigating the morphology of a species before embarking on studies where its size is an important factor. Several size factors might need to be considered, as mass is not always the best size factor. In addition, sexual dimorphism should be checked. Although *E. tenax* is a bigger fly than *E. pertinax*, the two species are not greatly dissimilar morphologically.

Chapter 3 - Water balance

3.1 Introduction

3.1.1 Water losses measurements - Techniques

In this chapter, the water losses of *E. tenax* and *E. pertinax* are investigated, looking at water losses in conditions of temperature and humidity reflecting those experienced in the field.

Water losses comprise a cuticular and a respiratory component, and authors have often tried to distinguish between the two. When an insect is resting, its cuticular transpiration usually predominates over its respiratory water loss. It ranges from 75% of total water loss, in tsetse flies, to 98.1%, in the ant *Camponotus vicinus* (Hadley 1994). In such a situation, it is justified to express water losses as cuticular permeability. However, respiratory losses increase when the insect becomes active. For example, they increase from 25% to 43% in tsetse flies and from 3% to 36% in the tenebrionid beetle *Eleodes armata* when they become active (Hadley 1994, Ahearn 1970). Ventilatory water losses also rise with metabolism when ambient temperature is high (Hadley 1994). Therefore, measured transpiration rates when the insect is active or when temperature is high are likely to include a substantial proportion of respiratory water loss. Thus, they do not represent cuticular permeability, and care has to be taken not to express them as cuticular permeability.

Various techniques have been used to estimate arthropods' water losses with more or less success. Also, the rates of water loss are expressed in a number of units. These units and techniques are described in Hadley (1994) and only an outline is given below.

A/ Units

A number of units have been used to express rates of water loss, and this makes comparison between studies very difficult. However, there has been some harmonisation of units recently. Hadley (1994) lists 3 main units for expressing cuticular transpiration:

- a proportion of the total mass of the animal per unit time (e.g. % body mass h^{-1}). It is suggested that this is only useful if the original water content is known. Vapour pressure deficit is not corrected for, so cross-experiment comparison is almost impossible.

- water loss rate, as the amount of water lost per unit surface area per unit time (e.g. mg cm^{-2} h^{-1}), presents the same problem for comparison.

- water loss per unit surface area per unit time per unit vapour pressure deficit (e.g. µg cm^{-2} h^{-1} $Torr^{-1}$, Torr is the same unit as mmHg), the most widely used unit now, includes a correction for vapour pressure. Correcting for the vapour pressure deficit allows the desiccating power of the air to be taken into account. Thus, experiments which have been conducted in different conditions of temperature and humidity can be readily compared. Data expressed that way can be converted into permeability with units of cm s^{-1}, if proper care is taken (as the conversion factor varies with temperature), the reciprocal of which is the cuticular resistance (in s cm^{-1}).

The last two units involve estimating the arthropods' surface area. Surface area is related to mass by the equation:

$$S = k\, m^{0.67}$$

where S is the surface area in cm^2, m is the mass in g and k is a constant. Both k and the exponent are species-specific and have ranged from 10.0 to 14.5 and from 0.62 to 0.72 respectively in insect studies (Noble-Nesbitt 1991). Some authors have assumed that the exponent is constant (0.67) and have estimated k by planimetric methods (Hadley 1994): the cuticle is cut into pieces that are then traced on squared paper;

or the various body parts are approximated to geometrical shapes (e.g. legs as cylinders) from which it is easy to calculate the surface area. Others have tried to establish species-specific formulae (Hadley 1994, Noble-Nesbitt 1991). In the present study, the exponent was assumed to be 0.67, and k was estimated (by approximation of body parts to geometrical shapes) to be 12 (based on two flies only). These surface area estimations are at best very inaccurate: the foldings and irregularities of the cuticle cannot be taken into account.

In addition, strictly speaking, the last two units refer to cuticular permeability and should not be used when respiratory water losses are involved. In this case, experimenters use amount of water per unit time (e.g. mg h^{-1}) or, to correct for size, amount of water per unit mass per unit time (e.g. mg g^{-1} h^{-1}) (e.g. Machin et al 1991, Quinlan and Hadley 1993).

B/ Gravimetric methods

Gravimetric methods, using sensitive microbalances, are easy and can give very valuable data if used sensibly but are also prone to a number of problems. It is assumed that if the arthropod is denied any access to water or food its mass loss is equivalent to its water loss once any excretion has been accounted for. The animal can be placed in a test chamber at prescribed conditions of humidity and temperature and weighed at regular intervals. This method avoids having to restrain the subject but may involve its disturbance at each weighing. Machin et al (1991) showed that intermittent weighing in the cockroach *Periplaneta americana* increases measured water loss rates by about 5-fold. To avoid repeated disturbance, the arthropod can be left on the balance pan and be weighed continuously. This involves restraining the animal, usually in a cage or bag made of mesh material. Restraining might itself lead to stress and increased water losses. In addition, the restraining material being in close contact with the animal, a boundary layer might be created which lowers the humidity gradient between the arthropod's surface and air, thus reducing water losses. The same problem might arise when the experiment is run in still air as humidity gradients might build up in the "container". Also, depending on the size of the test chamber relative to the subject, humidity levels might increase noticeably. One way around these

problems is to circulate the air with a fan or to pump air through the chamber, controlling the humidity of the air entering.

Gravimetry measures the *net* water lost by the animal (i. e. water lost - metabolic water produced). It does not distinguish between cuticular and respiratory water losses, but if the animal is resting, respiratory water losses are small compared to cuticular water losses. Thus, measured losses can be assumed to be equivalent to cuticular transpiration. However, this is only valid at temperatures at which the metabolism and thus ventilatory losses are minimal (Hadley 1994). Some authors have blocked spiracles, mouth and anus of their subjects with wax, with more or less success, in order to eliminate respiratory and excretory losses. In fact, in some studies, water losses were increased after blockage, perhaps because of some damage done during the procedure (Hadley 1994). Several authors have used water losses of dead insects to estimate cuticular transpiration (e.g. Loveridge 1968a). However, as Hadley (1994) points out, this is better avoided as water losses of dead animals are greater than those of live ones, even when the spiracles are blocked. Hadley suggests that the reasons behind this phenomenon are the loss of some active cuticular control mechanism and/or damage caused by handling.

Continuous mass loss recording in quiescent animals can show the cycles of ventilation (e.g. Machin et al 1991) and can help distinguish between the two components of water losses. The periods of slow water loss are assumed to correspond to spiracle closure and represent cuticular transpiration. The periods of fast water loss correspond to the opening of spiracles and gas exchange. By subtracting one from the other, the proportion of respiratory water loss can be estimated (Machin et al 1991).

C/ Electronic moisture sensing

This technique measures the humidity of the air exiting the test chamber and thus enables the experimenter to determine how much water is transpired by the animal. This records the *gross* water loss and is valuable to estimate how much water is lost, but does not reveal much about the water balance of the subject as the production of metabolic water is not taken into account. This can be rectified if CO_2 production is measured at the same time, as metabolic water production can then be calculated. The beauty of this method is that it allows comparison of water

losses in resting and active (even flying) animals. It reflects natural conditions more accurately. Louw and Hadley (1985) have used it to show that although water losses increase dramatically in flying honeybees, they are almost compensated by metabolic water production. Likewise, Nicolson and Louw (1982), using O_2 consumption, have shown that flying carpenter bees *Xylocopa capitata* remain in water balance as long as ambient temperature does not exceed 27 °C.

Coupling gravimetry with CO_2 emission sensing helps refine the investigation of respiratory transpiration and its control. This technique has been employed by Lighton (1992) with ants. He showed that peaks of CO_2 emission correspond to peaks of water loss. Other authors (e.g. Quinlan and Hadley 1993) have used electronic sensing to measure water losses, oxygen consumption and carbon dioxide excretion to study discontinuous carbon dioxide release and the regulation of ventilatory cycles.

D/ Radioisotopes

The use of radioisotopes, usually tritiated water, allows the investigation of water balance in free-ranging animals and in subjects which are too small (such as mites) for gravimetric methods. Here, both active and passive water exchanges are involved, but it is possible to calculate the amount of water transpired by the arthropod for a specified period (Hadley 1994). Again, *gross* water losses are measured, and these include both cuticular and ventilatory losses.

The animal is either injected with tritiated water of known specific activity or is allowed to take up tritiated water from food or from an atmosphere in equilibrium with a tritiated solution of sufficiently high water activity. This requires a preliminary experiment to determine the period needed for the haemolymph to come to equilibrium with the tritiated water, but it then considerably reduces the stress for the subjects. Then, either samples of haemolymph are withdrawn and their isotopic activity determined or, alternatively, the animal is killed and the isotopic activity of its body measured. From the data obtained the amount of water lost can be estimated (Hadley 1994, Wolf et al 1996).

Results obtained with this technique tend to be slightly higher than those obtained by gravimetry (Hadley 1994). The possible reasons put

forward for this are: the measured water losses are gross losses; tritium might not be well mixed with intracellular water at the beginning of the experiment, so some of the decrease in haemolymph activity reflects tritium entering cells as well as tritium being lost to the outside; some tritium becomes linked to some organic materials and is lost from the pool.

Nevertheless, this technique seems very promising for studies where the water balance of the arthropod is of interest. It could even be used in the field, as long as the animals can be recaptured. For example, it could be envisaged to study water loss in overwintering *E. tenax*.

The use of radioisotopes can also be coupled to a flow-through system. The radioactivity of the air (due to water loss and/or CO_2 excretion, depending which radioisotopes are used) leaving the test chamber is measured: water losses and CO_2 emission can thus be estimated (e.g. Croghan et al 1995, Wolf et al 1996).

E/ Direct measurements of cuticular transpiration

All the methods described above measure both cuticular and respiratory water losses. It can be of interest in some situations to estimate cuticular transpiration on its own.

Techniques for direct cuticular transpiration measurement have been developed (Hadley 1994, Hadley et al 1989, Nicolson et al 1984) . A capsule of known diameter is sealed on the animal's cuticle (with wax). Air is circulated through the capsule and takes in the transpired moisture. The amount of transpired moisture is then determined gravimetrically, electronically (humidity sensors), or radioisotopically (with animals having previously been injected with a radioisotope) from the air exiting the capsule. Nicolson et al (1984) used the radioisotope method with the beetle *Onymacris plana* and found that the water loss rate were about 53% of those determined gravimetrically (see also Table 3.34).

These methods have the advantage of using live animals with intact openings and of determining direct cuticular transpiration across a known surface area (if irregularities of the cuticle are ignored). The methods described above use very inaccurate estimates of surface area.

Some experimenters have also measured transpiration across excised pieces of cuticle. This technique is becoming more accurate but still

involves the risk of damaging the cuticle during the dissection. Indeed, cuticular permeabilities measured this way are often higher than those measured gravimetrically. Nevertheless, Hadley (1994) argues that this is a promising technique to investigate the role of lipids in cuticular permeability.

3.2 Materials and methods

The purpose of this study was to investigate the overall water balance problems that *E. tenax* and *E. pertinax* might face, without distinguishing between cuticular and respiratory water losses. The experimental conditions had to reflect field conditions and animal disturbance had to be kept to a minimum. The radioisotope technique would have been the method of choice, with the possibility of trying to use it in the field in overwintering flies. However, it turned out that it was impossible to withdraw any haemolymph from winter flies, probably because of dehydration. Because of time constraints, it was not possible to use radioisotopes in the summer only and another technique for both summer and winter to allow comparison. Therefore, it was decided to rely on gravimetry for both seasons, but this posed a further problem. The electronic balance available (Perkin-Elmer AD-4 autobalance) could not be used at temperatures lower than 15 °C (manufacturer recommendations), too high to reflect winter conditions.

As will be described below, a series of experiments was run with restrained flies in the balance in order to obtain some continuous records of mass loss. In another series, flies were left free in various conditions of temperature and humidity and were weighed at the beginning and end of the experiment to minimise the disturbance.

In all experiments, live flies were starved (but had access to water) for at least 12 hours, to minimise the risk of defecation. They were kept in plastic boxes at 5 ± 1°C and 96.5 ± 0.4 % RH (in a refrigerator).

Controls were run with flies freshly killed in a killing bottle containing ethyl acetate.

3.2.1 Continuous mass loss recording

Flies were briefly anaesthetised with CO_2 and enclosed in a small (just big enough to enclose a fly) pre-weighed bag made of nylon net. The bag with the fly inside was placed on the weighing pan of the electronic balance, in the chamber of which the required humidity was achieved by placing pots (5 cm diameter) filled with distilled water. The humidity in the chamber was measured using the "wiggly wire" technique (Unwin 1980). Metallic wires are shaped in a series of loops into which droplets of a specified solution (here saturated potassium acetate 30%) are exposed to air and allowed to equilibrate. The concentration of the droplets is then determined using a pocket refractometer. A drop of the liquid is placed in the refractometer and concentration is read. The humidity of the air is then read from a humidity/concentration calibration curve. In the present work, the experiments were run at two humidities: 46 ± 2 % and 96 ± 2 % were used.

Mass was recorded every five minutes for the first quarter of an hour and every ten minutes for the remaining hour and three quarters.

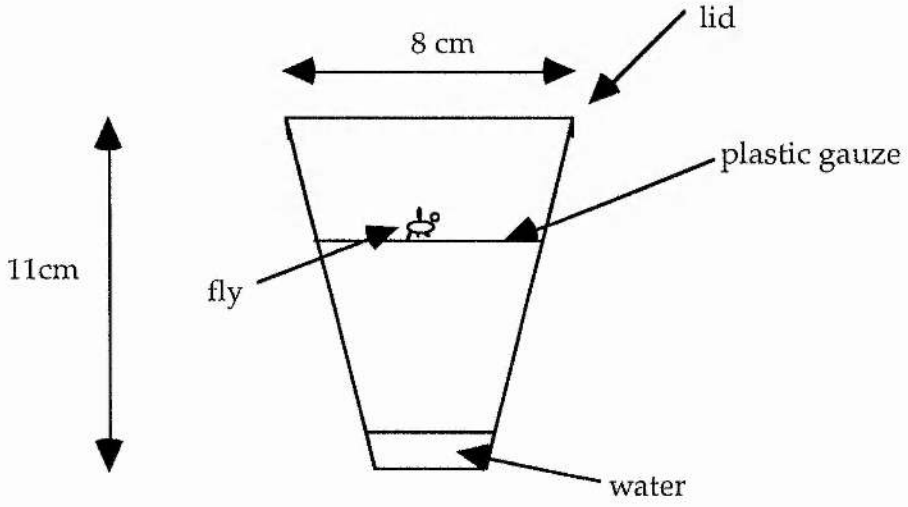
After two hours, the flies were taken out of the balance, and the bag was weighed again to check for any increase in mass which might indicate some excretion by the fly. Excretion would also have shown by a sudden drop in mass between two successive records. No problem was encountered with this.

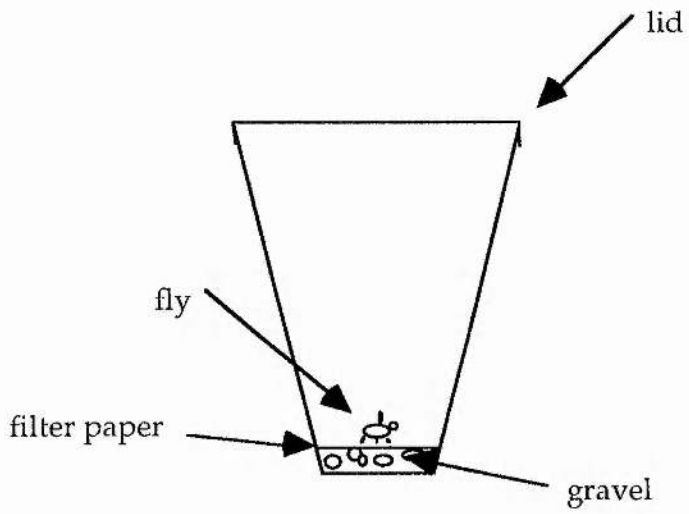
Water loss rates were calculated from the gradient of the curve of mass against time. In some cases, the rate of water loss was higher at the beginning of the experiment and only became constant after a while (within the first hour); only the straight part of the curve was used (see also Figure 3.12).

At high relative humidity, there were problems with this experiment, as water seemed to condense on the bag material. Thus, empty bags were tested. These were left in the balance for several hours at high humidity. They were then taken out to mimic placing a fly in them, replaced in the balance, and their mass recorded every 5 minutes. Mass was found to increase and only stabilised after 30 to 60 minutes, depending on the bag. It is thus clear that moisture condenses on these bags.

3.2.2 Unrestrained flies

To avoid restraining the flies (minimum stress) and the problem of water condensation on the bag material, and to be able to run the experiments at low ambient temperature, another experiment was designed. Plastic pots (8 cm diameter, 11 cm high) were set up with either water or gravel at the bottom (see diagram below). In the pots with water, a plastic gauze was fixed about two thirds up and acted as a "floor" for the fly placed in the pot. In pots with gravel, a piece of white filter paper was placed on top of the gravel to check for traces of defecation. Only rarely did the flies defecate and these results were discarded.





Relative humidity (measured with the "wiggly" wire technique) was 46 ± 2 % in the pots with gravel and 96 ± 2 % in the pots with water.

Four conditions were selected:

- high relative humidity, low temperature (7 ± 2 °C)
- high relative humidity, high temperature (20 ± 2 °C)
- low relative humidity, low temperature
- low relative humidity, high temperature

The pots were either left on a laboratory bench (high temperature) or placed in a refrigerator (low temperature) at least one hour before the experiment started.

Flies were cooled down, briefly anaesthetised with CO_2, weighed (in the electronic balance) and then placed in a pot which was opened and closed very quickly and was replaced in its temperature environment (i. e. bench or refrigerator) immediately. Only one fly was allowed per pot. The pots and flies were then left undisturbed for two hours. This time period was selected because it had previously been shown with the continuous mass recording that the flies reach a steady water loss after about an hour. A longer period might have given more accurate results as the first hour sometimes shows a higher rate, so a few experiments were run for four hours. They did not lead to consistently lower or higher water loss rates and, because of time constraints, the two hour period was selected. At the end of the experiment, the flies were caught and anaesthetised briefly to be weighed again. Pots from the high temperature condition were placed

in ice for a few minutes to cool the flies down before they were caught. Water loss rate was calculated by subtracting the final mass from the initial.

It is clear that the way this experiment was conducted was not ideal for maximum accuracy. The duration was sometimes extended by three or four minutes for cooling the flies down. Also , the level of activity was not recorded, but was certainly higher in flies kept at high temperature. In addition, placing the flies in the pots probably disturbed the humidity level inside which might have taken some time to reach its assumed value again. Nevertheless, it provided a simple way to investigate water losses in "free" animals, in conditions mimicking those that might be encountered in the field (the temperatures are close to those experienced in summer and winter by the flies, as are the relative humidities). In fact the most representative conditions were high humidity and low temperature (which mimicked overwintering conditions), and low humidity and high temperature (which corresponded to summer field conditions). The other two conditions were used so that the effect of changing only one parameter (i.e. either temperature or humidity) could be studied.

3.2.3 Calculations, statistics and graphic representation

The water loss rates calculated include both cuticular and respiratory losses, especially in the experiments at high temperature as the flies were quite active, even flying from time to time. Therefore, it seemed unjustified to use units for cuticular permeability to express these rates. Thus, the amount of water lost (in mg) per unit time (h) (water loss rate, WLR) and the amount of water lost (in mg) per unit of fly's mass (g) per unit time (h) (specific water loss rate, SWLR) were used. Water loss rate gives an idea of how much water is actually lost, whereas specific water loss rate takes into account the mass of the animal. Data expressed as water loss rate could be compared with studies that have investigated the proportion of total water loss represented by respiratory water losses, as the same units are used. However, most data in the literature refer to cuticular permeability and are given as water lost per unit surface area per unit time per unit vapour pressure deficit (e.g. $\mu g\ cm^{-2}\ h^{-1}\ Torr^{-1}$). In the present study, it could be justified to use this unit where the flies were

quiescent and temperature was not too high as respiratory water losses are likely to be small compared to cuticular water losses. Therefore, water loss rates in the condition of low temperature (both high and low humidity) were expressed that way to allow comparison with other species.

All data sets were tested for normality and, if necessary, transformed before using any parametric test. All the tests were carried out using "Minitab" version 8.2 on an Apple Macintosh.

Regression was used to analyse the effects of continuous variables on one another. The equation of the best fitted line is given either in the text or in the figure legend; n, p and R^2 values are given in the text.

The general linear model was employed (because of unequal sample sizes) to investigate the effect of a number of variables (non-continuous and continuous, in which case a covariance analysis was done) on another.

To represent graphically the effect of one variable on another when other factors are also involved requires a control for these other factors. When considering the effect of a continuous variable (e.g. mass) on a second continuous variable (e.g. water loss rate), this has been done by obtaining the residuals of the covariance analysis for the other factors on the second variable. The residuals represent the values of the second variable when other factors have been controlled for. The residuals were then plotted against the continuous variable. This could also have been done in the case of discreet variables (e.g. humidity and temperature conditions), but this was avoided because it makes scales meaningless and comparisons impossible. In addition, column graphs can include several variables (e.g. water loss rate at the four humidity and temperature conditions for males and females of a species) which makes using residuals (or fitted means = mean for each variables when the others have been controlled for) not an option. Thus, on the column graphs the "actual" values are plotted. The consequence is that, sometimes, a statistically significant result appears non significant on the graph, and this should be kept in mind.

3.3 Results

3.3.1 Effect of restraining

Some flies were restrained in small nylon mesh bags in order to obtain continuous records of mass loss and some measurements of water loss in "resting" flies. Because the balance could not be used at temperatures lower than 15 °C, only conditions of high and low humidity at high temperature were tested.

Covariance analyses were carried out for mass, condition and restrained/unrestrained (state) factor on the rate of water loss and on the specific rate of water loss for *E. tenax* in the winter. For *E. tenax* and *E. pertinax* in the summer, the experiments were only run under one condition (low relative humidity and high temperature), so condition did not need to be controlled for. However sex had to be controlled for. When controlled for, sex was shown to be a non significant factor and was thus omitted from the analyses.

A/ *E. tenax*

Tables 3.1 and 3.2 show the results of the analyses for live flies in the winter. The effect of mass and condition will be discussed later, as the purpose of this section is to concentrate on the effect of restraining flies on water loss. Clearly, restrained live flies in winter have lower rates of water loss and specific rates of water loss than unrestrained flies. This is also shown in Figure 3.1a & b.

Table 3.1 Covariance analysis on the rate of water loss for mass, condition state of restraining in live *E. tenax* in winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.051	0.102	0.102	0.32	0.576
Condition	1	6.606	9.931	9.931	30.68	<0.001
State	1	5.482	5.482	5.482	16.93	<0.001
Error	81	26.221	26.221	0.324		
Total	84	38.359				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Restrained	1.09	0.09
Unrestrained	1.65	0.11

Table 3.2 Covariance analysis on the specific rate of water loss for mass, condition state of restraining in live *E. tenax* in winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	205.84	185.21	185.21	7.01	0.010
Condition	1	495.26	709.42	709.42	26.83	<0.001
State	1	327.13	327.13	327.13	12.37	0.001
Error	81	2141.38	2141.38			
Total	84	3169.61				

Fitted mean for the specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Restrained	9.60	0.79
Unrestrained	13.94	0.96

Again, Tables 3.3 and 3.4 and Figure 3.1c & d show that live unrestrained *E. tenax* in summer lose water faster than restrained flies.

Table 3.3 Covariance analysis on the rate of water loss for mass and state of restraining in live *E. tenax* in summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.665	0.663	0.663	0.66	0.422
State	1	6.338	6.338	6.338	6.34	0.017
Error	30	29.995	29.995	0.100		
Total	32	36.999				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Restrained	1.64	0.22
Unrestrained	2.53	0.28

Table 3.4 Covariance analysis on the specific rate of water loss for mass and state of restraining in live *E. tenax* in summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	154.92	155.22	155.22	2.00	0.168
State	1	619.43	619.43	619.43	7.97	0.008
Error	30	2331.57	2331.57	77.72		
Total	32	3105.92				

Fitted mean for the specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Restrained	15.42	1.97
Unrestrained	24.29	2.45

Because of time constraints, dead *E. tenax* were only tested in winter and only in the condition of low relative humidity and high temperature. Again the results shown in Tables 3.5 and 3.6 and in Figure 3.1e & f indicate that unrestrained dead flies lose water faster than restrained ones.

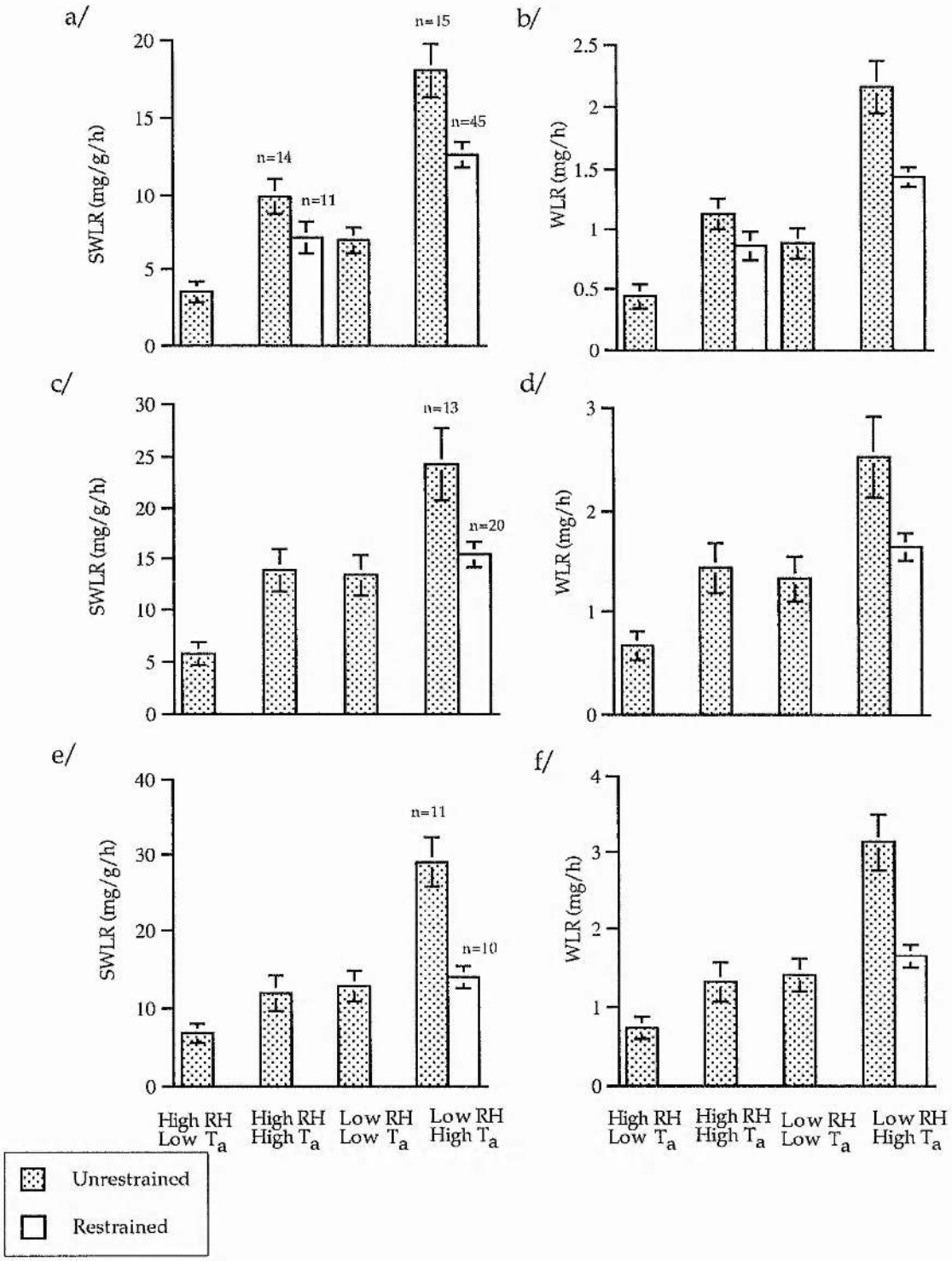


Fig. 3.1 Comparison of water loss in unrestrained and restrained *E. tenax*

a/ Specific water loss rate vs conditions in live flies in winter ($p<0.001$)
b/ Water loss rate vs conditions in live flies in winter ($p<0.001$)
c/ Specific water loss rate vs condition in live flies in summer ($p=0.008$)
d/ Water loss rate vs condition in live flies in summer ($p=0.017$)
e/ Specific water loss rate vs condition in dead flies in winter ($p=0.002$)
f/ Water loss rate vs condition in dead flies in winter ($p=0.001$)

Table 3.5 Covariance analysis on the rate of water loss for mass and state of restraining in dead *E. tenax* in winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.052	0.950	0.950	1.09	0.310
State	1	12.959	12.959	12.959	14.90	0.001
Error	18	15.653	15.653	0.870		
Total	20	28.665				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Restrained	1.57	0.30
Unrestrained	3.24	0.29

Table 3.6 Covariance analysis on the specific rate of water loss for mass and state of restraining in dead *E. tenax* in winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	204.28	9.76	9.76	0.13	0.723
State	1	983.77	983.77	983.77	13.03	0.002
Error	18	1359.51	1359.51	75.53		
Total	20	2547.56				

Fitted mean for the specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Restrained	14.27	2.84
Unrestrained	28.79	2.70

B/ *E. pertinax*

Tables 3.7 and 3.8 show that live restrained *E. pertinax* lose water at the same rate as unrestrained flies. However, Figure 3.2a & b suggest that, even though the effect of restraining is not statistically significant, there is a trend towards unrestrained flies losing water faster than restrained ones.

Table 3.7 Covariance analysis on the rate of water loss for mass and state of restraining in live *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.168	0.212	0.212	1.37	0.262
State	1	0.278	0.278	0.278	1.79	0.202
Error	14	2.174	2.174	0.1555		
Total	16	2.621				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Restrained	1.18	0.18
Unrestrained	1.46	0.11

Table 3.8 Covariance analysis on the specific rate of water loss for mass and state of restraining in live *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	160.73	139.54	139.54	4.99	0.042
State	1	65.06	65.06	65.06	2.33	0.150
Error	14	391.67	391.67	27.98		
Total	16	617.46				

Fitted mean for the specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Restrained	17.88	2.37
Unrestrained	22.20	1.53

The results for dead *E. pertinax* are similar to those for live flies, i.e. although Figure 3.2c & d show that dead unrestrained flies have a tendency to lose water faster than dead restrained flies, this result is not statistically significant (Tables 3.9 and 3.10).

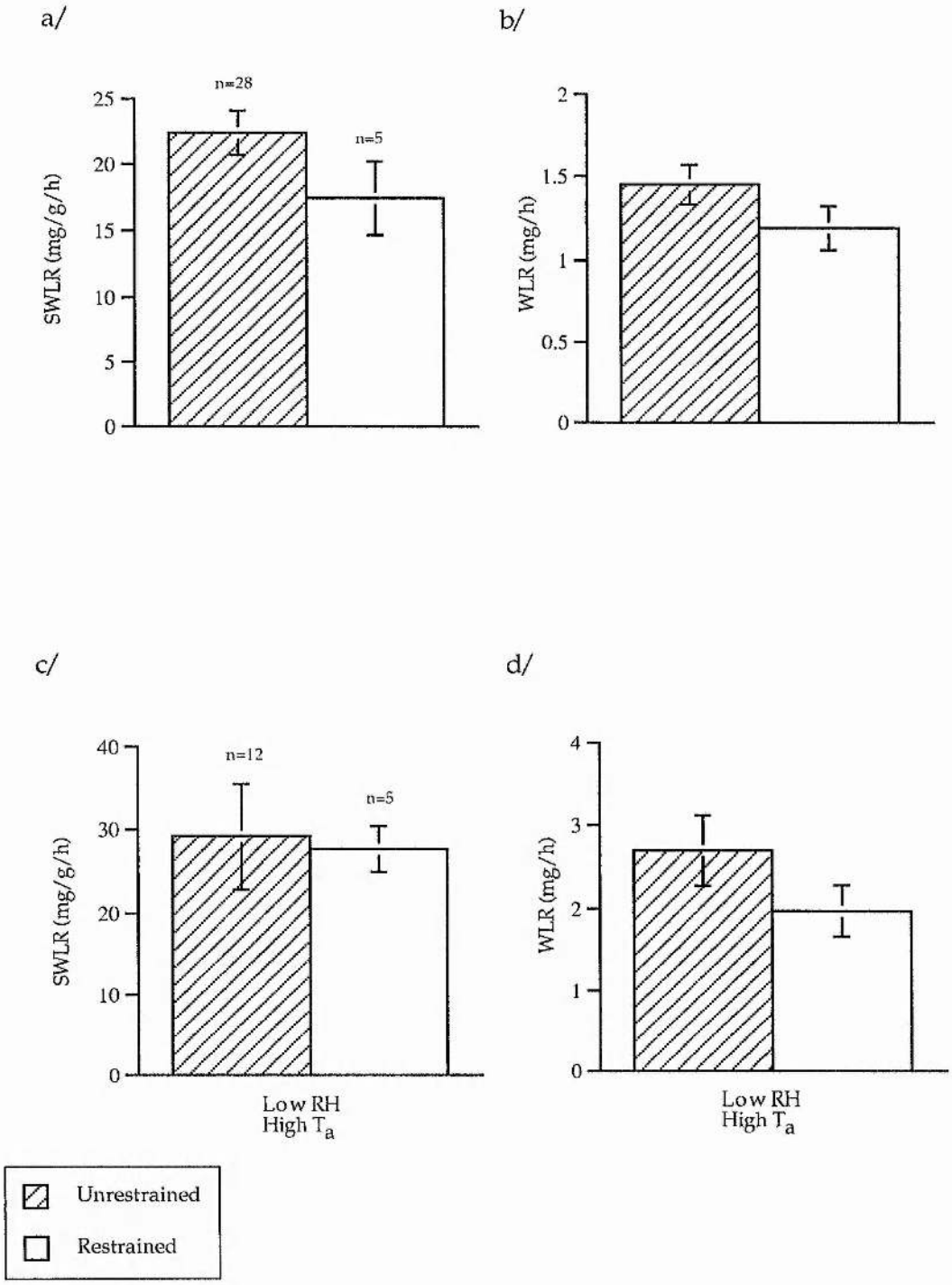


Fig. 3.2 Comparison of water loss at low humidity and high temperature in unrestrained and restrained *E. pertinax*

a/Specific water loss rate in live flies (p=0.150)
b/Water loss rate in live flies (p=0.202)
c/ Specific water loss rate in dead flies (p=0.254)
d/ Water loss rate in dead flies (p=0.376)

Table 3.9 Covariance analysis on the rate of water loss for mass and state of restraining in dead *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.771	0.160	0.160	0.21	0.661
State	1	0.686	0.686	0.686	0.89	0.376
Error	7	5.372	5.372	0.767		
Total	9	6.828				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Restrained	2.04	0.42
Unrestrained	2.63	0.42

Table 3.10 Covariance analysis on the specific rate of water loss for mass and state of restraining in dead *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	266.56	385.24	385.24	4.77	0.065
State	1	124.61	124.61	124.61	1.54	0.254
Error	7	564.88	564.88	80.70		
Total	9	956.05				

Fitted mean for the specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Restrained	24.42	4.28
Unrestrained	32.36	4.28

C/ Summary of section 3.3.1

When these experiments were carried out, they were expected to show that live restrained flies ("resting" flies) have lower rates of water loss than live unrestrained flies ("active" flies). The experiments on dead flies acted as controls, and it was anticipated that the rate of water loss in restrained and unrestrained flies would be similar. Live *E. tenax* restrained flies do lose water more slowly than unrestrained ones: this could reflect the effect of activity and the increased respiratory water loss,

and was as expected. There is a tendency towards a similar result in *E. pertinax*, but this is not statistically significant. However, restrained dead *E. tenax* also lose water more slowly than unrestrained flies (again, the same tendency, although not significant, is observed in *E. pertinax*). This suggests that restraining flies in a nylon mesh bag does alter the measured rate of water loss. The mechanisms by which this might happen will be discussed later, but in the light of these results, the comparison between restrained and unrestrained flies was abandoned. Thus, the results which follow are for unrestrained flies only.

3.3.2 Seasonal effect on the water loss of *E. tenax*

Although female *E. tenax* overwinter in habitats where humidity is high (usually above 90%, see Chapter 6), they do not have access to drinking water unless they become active (or sometimes when the humidity becomes very high and water is seen dripping from the walls of the overwintering site). In summer, active flies have access to water from nectar, possibly from other drinking sources, and from metabolic water production. This section looks at the water loss in *E. tenax* in winter and summer. Only females were considered because only females overwinter.

Due to time constraints, not enough dead female *E. tenax* were tested in the summer to make any analysis of dead flies meaningful. Thus, the data presented are for live females only.

Covariance analyses were carried out for mass, condition and season on the rate of water loss and the specific rate of water loss (Tables 3.11 and 3.12). These results and Figure 3.3a & b show that the specific rate of water loss of *E. tenax* in winter is lower than in summer. The same trend is present when considering the rate of water loss, but is not quite statistically significant (Table 3.11 and Figure 3.3c & d). The effect of mass and condition will be discussed later.

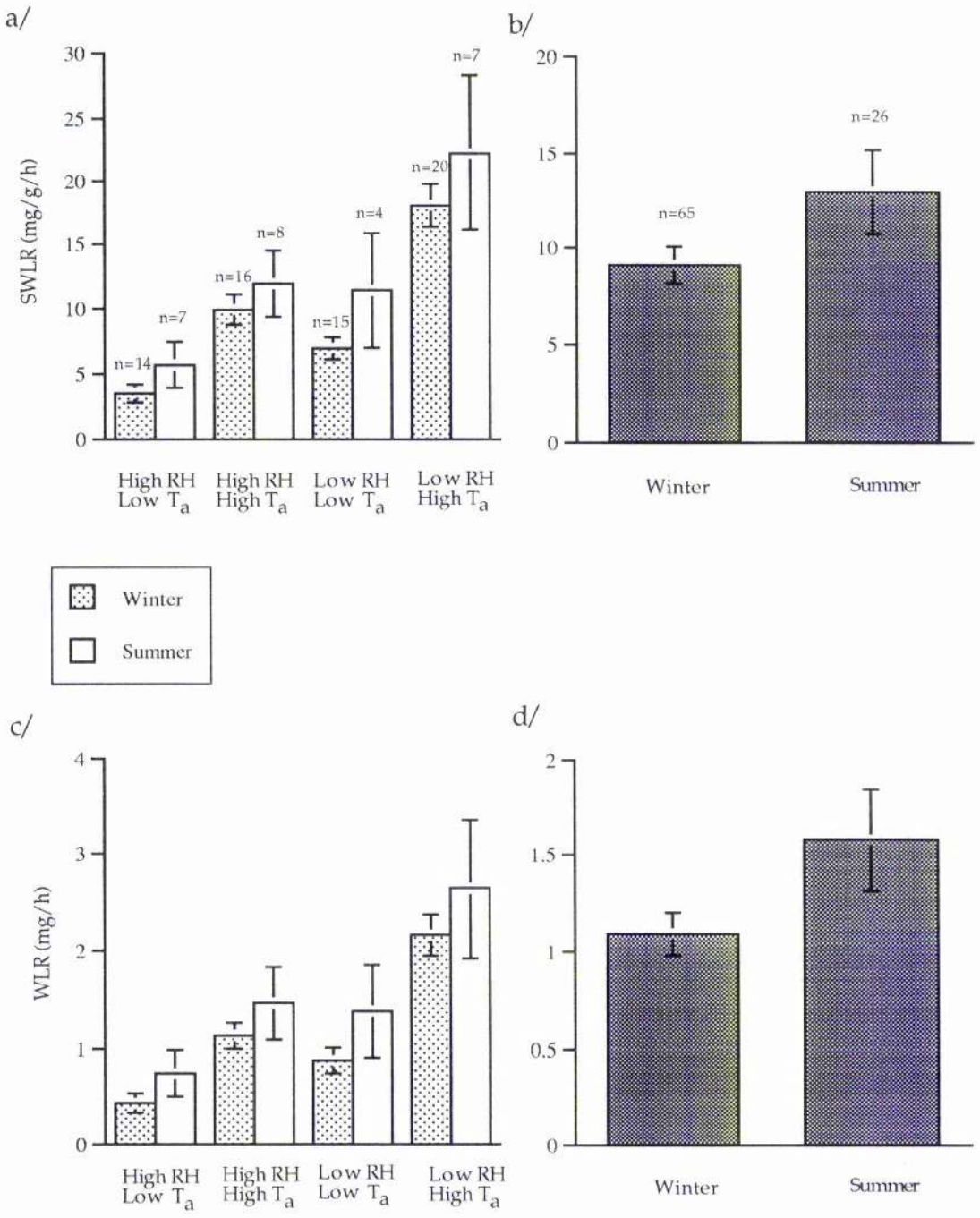


Fig. 3.3 Effect of season on water loss in females *E. tenax*

a/ Specific water loss rate vs conditions
b/ Specific water loss rate vs season
c/ Water loss rate vs conditions
d/ Water loss rate vs season

Table 3.11 Covariance analysis on the rate of water loss for mass, condition and season in *E. tenax* (females)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	3.178	2.919	2.919	4.80	0.031
Condition	3	41.327	39.851	13.284	21.82	<0.001
Season	1	2.091	2.091	2.091	3.44	0.067
Error	85	51.736	51.736	0.609		
Total	90	98.332				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Winter	1.17	0.10
Summer	1.51	0.16

Table 3.12 Covariance analysis on the specific rate of water loss for mass, condition and season in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.32	0.01	0.01	0.00	0.989
Condition	3	2979.87	2869.94	956.65	23.07	<0.001
Season	1	166.52	166.52	166.52	4.02	0.048
Error	85	3524.95	3524.95	41.47		
Total	90	6671.66				

Fitted mean for the specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Winter	9.63	0.81
Summer	12.68	1.28

The size of winter flies was also compared with that of summer flies with a covariance analysis on mass for thoracic width and season (Table 3.13). It is clear that, in winter, flies of the same thoracic width are lighter than in summer. This result could partly be linked to differences between food contents of the gut, (but summer flies were starved for at least 12

hours before the experiment), or to fat reserves, and certainly, to dehydration. That winter flies are dehydrated was confirmed by the impossibility of withdrawing haemolymph from them .

Table 3.13 Covariance analysis on mass for season and Th_w in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	8610.3	9568.1	9568.1	26.77	<0.001
Season	1	2217.5	2217.5	2217.5	6.20	0.015
Error	81	28955.0	28955.0	357.5		
Total	83	39782.8				

Fitted mean for mass

	Mean (mg)	Stdev (mg)
Winter	117.9	2.39
Summer	129.9	4.15

Thus, *E. tenax* in winter are probably dehydrated and lose water at a lower rate than in summer.

3.3.3 Effect of sex, size and condition

Mass was used as the size factor as thoracic width was not found to be a significant predictor of water loss.

A/ *E. tenax*

Because of the season effect described above, the effect of size and condition on water loss was investigated separately in winter and summer flies. A potential sex effect was investigated in summer flies only.

a/ Summer

Covariance analyses of the effect of mass, condition and sex on the water loss rate were carried out in live (Tables 3.14 and 3.15) and dead (Tables 3.16 and 3.17) flies.

Tables 3.14 and 3.15 show that there is no difference in the rate of water loss in live female and male *E. tenax*. In addition, there is a positive relationship between water loss rate and mass but none between specific water loss rate and mass in live flies. Thus, large flies lose water more rapidly than small ones, but when water loss per unit mass is considered,

small and large flies lose water at the same rate. The relationship between mass and the residuals from the covariance analysis for condition on water loss rate is shown in Figure 3.4a. Although the interaction between sex and mass is not a significant factor (Tables 3.14 and 3.15), mass only becomes a significant predictor when this interaction is included. Thus, in Figure 3.4a the sexes are separated to reflect the effect of this interaction (males: n=26, R^2=0.36, mass p=0.002; females: n=26, R^2=0.014, mass p=0.587).

Condition is a very significant factor. The rate of water loss as well as the specific rate of water loss vary with temperature and humidity conditions (Figure 3.5a & c). An increase in temperature at a constant humidity or a reduction of humidity at a constant temperature both lead to a rise in transpiration. Water loss rates are the lowest at low temperature and high humidity and the highest at high temperature and low humidity. This reflects the changing desiccating effect of the air as well as increased activity at high temperature of live flies.

Table 3.14 Covariance analysis on the rate of water loss for mass, condition, sex and the interaction between sex and mass in live *E. tenax* in summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	1.983	3.693	3.693	4.19	0.047
Condition	3	23.624	23.927	7.976	9.04	<0.001
Sex	1	0.266	1.066	1.066	1.21	0.277
Sex*Mass	1	1.469	1.469	1.469	1.67	0.203
Error	45	39.682	39.682	0.882		
Total	51	67.024				

Fitted mean for rate of water loss

	Mean (mg/h)	Stdev (mg/h)
High RH Low T_a	0.75	0.28
High RH High T_a	1.64	0.29
Low RH Low T_a	1.49	0.30
Low RH High T_a	2.65	0.28

Table 3.15 Covariance analysis on the specific rate of water loss for mass, condition, sex and the interaction between sex and mass in live *E. tenax* in the summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	83.30	1.58	1.58	0.02	0.882
Condition	3	2229.65	2262.78	754.26	10.66	<0.001
Sex	1	59.43	9.62	9.62	0.14	0.714
Sex*Mass	1	28.44	28.44	28.44	0.40	0.529
Error	45	3183.82	3183.82	70.75		
Total	51	5584.64				

Fitted mean for specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
High RH Low T_a	6.45	2.48
High RH High T_a	14.86	2.64
Low RH Low T_a	13.52	2.64
Low RH High T_a	24.97	2.50

Tables 3.16 and 3.17 show that there is no difference in the rate of water loss of dead males and females. However, there is an almost significant interaction between sex and mass, which indicates that the relationship of water loss rate and specific water loss rate with mass is not the same in females and males. Figure 3.4c shows that males have a positive relationship between their water loss rate and mass whereas females do not (males: n=15, R^2=0.23, mass p=0.040; females: n=8, mass p=0.807). Thus, large males lose water faster than small males. Figure 3.4b shows the negative relationship between the specific water loss rate and mass in females (n=8, R^2=0.39, mass p= 0.016); there is no relationship between the specific water loss rate and mass in dead males (n=15, mass p=0.592). Therefore, small dead females lose water faster per unit mass than large ones. Again condition is a very significant factor (Figure 3.5a & c). Here, the difference in water loss rate at various conditions of temperature and humidity reflects the difference in the desiccating effect of air only, as the flies are dead.

Table 3.16 Covariance analysis on the rate of water loss for mass, condition, sex and the interaction of sex and mass in dead *E. tenax* in the summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	1.232	0.592	0.592	3.77	0.070
Condition	3	10.710	8.618	2.873	18.30	<0.001
Sex	1	0.426	0.483	0.483	3.07	0.099
Sex*Mass	1	0.691	0.691	0.691	4.40	0.052
Error	16	2.512	2.512	0.157		
Total	22	15.571				

Fitted mean for rate of water loss

	Mean (mg/h)	Stdev (mg/h)
High RH Low T_a	0.58	0.18
High RH High T_a	1.27	0.22
Low RH Low T_a	1.71	0.19
Low RH High T_a	2.36	0.17

Table 3.17 Covariance analysis on specific the specific rate of water loss for mass, condition, sex and the interaction of sex and mass in dead *E. tenax* in the summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	2.21	18.47	18.47	1.54	0.233
Condition	3	766.13	645.91	215.30	17.90	<0.001
Sex	1	7.76	42.58	42.58	3.54	0.078
Sex*Mass	1	51.43	51.43	51.43	4.28	0.055
Error	45	192.41	192.41	12.03		
Total	51	1019.95				

Fitted mean for specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
High RH Low T_a	4.50	1.56
High RH High T_a	10.11	1.95
Low RH Low T_a	15.15	1.64
Low RH High T_a	19.51	1.48

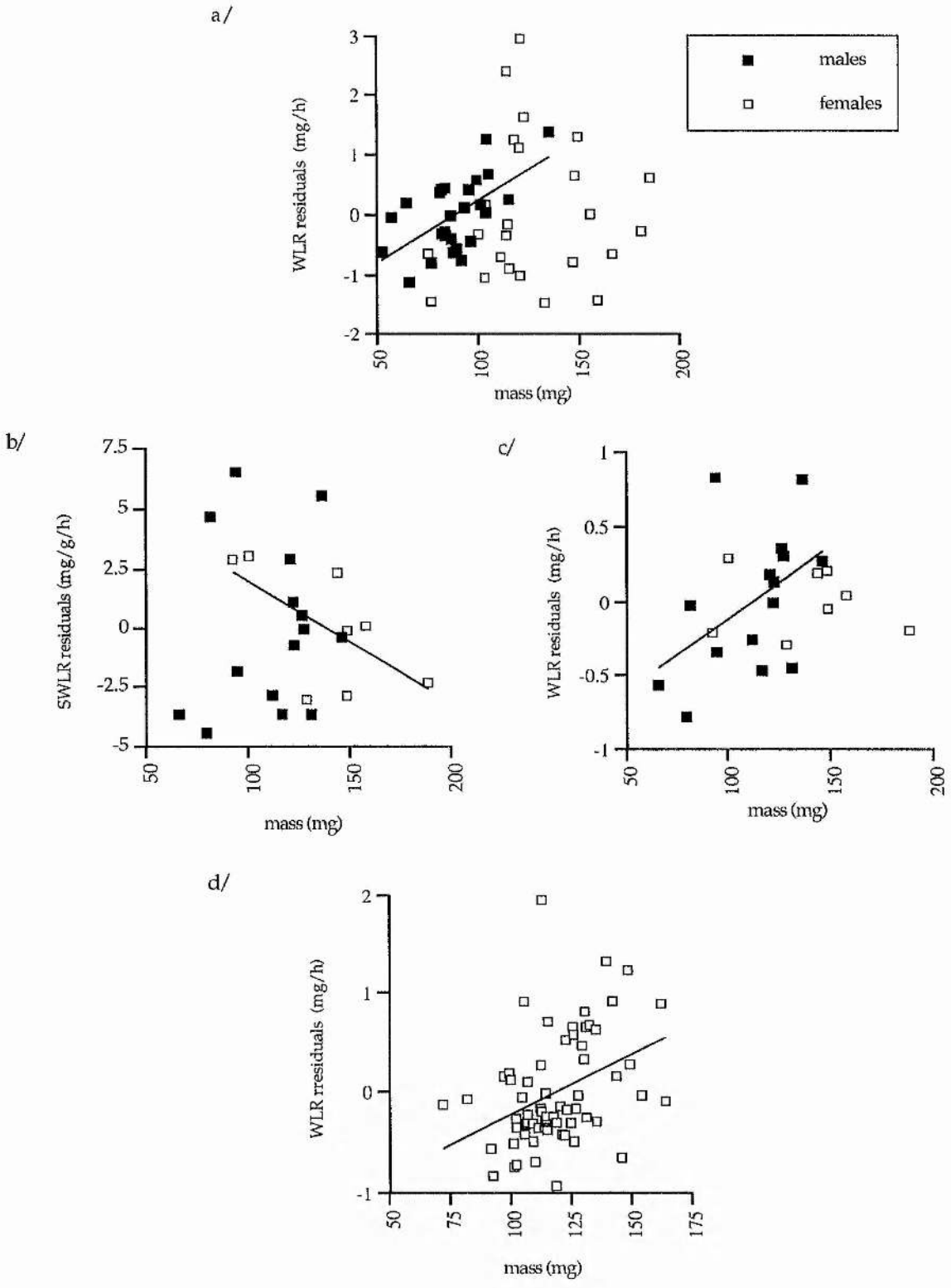


Fig. 3.4 Relationships between mass and water loss rate and specific water loss rate in *E. tenax* (condition has been controlled for so the residuals of the glm analyses are plotted)

a/ Live flies in summer - WLR vs mass; males: y= 0.021x - 1.82, r^2=0.36
b/ Dead flies in summer - SWLR vs mass; females: y = -0.052x + 7.212, r^2 = 0.39
c/ Dead flies in summer - WLR vs mass; males: y = 0.010x - 1.102, r^2 = 0.23
d/ Live flies in winter (females) - WLR vs mass: y = 0.012x - 1.42, r^2 = 0.15

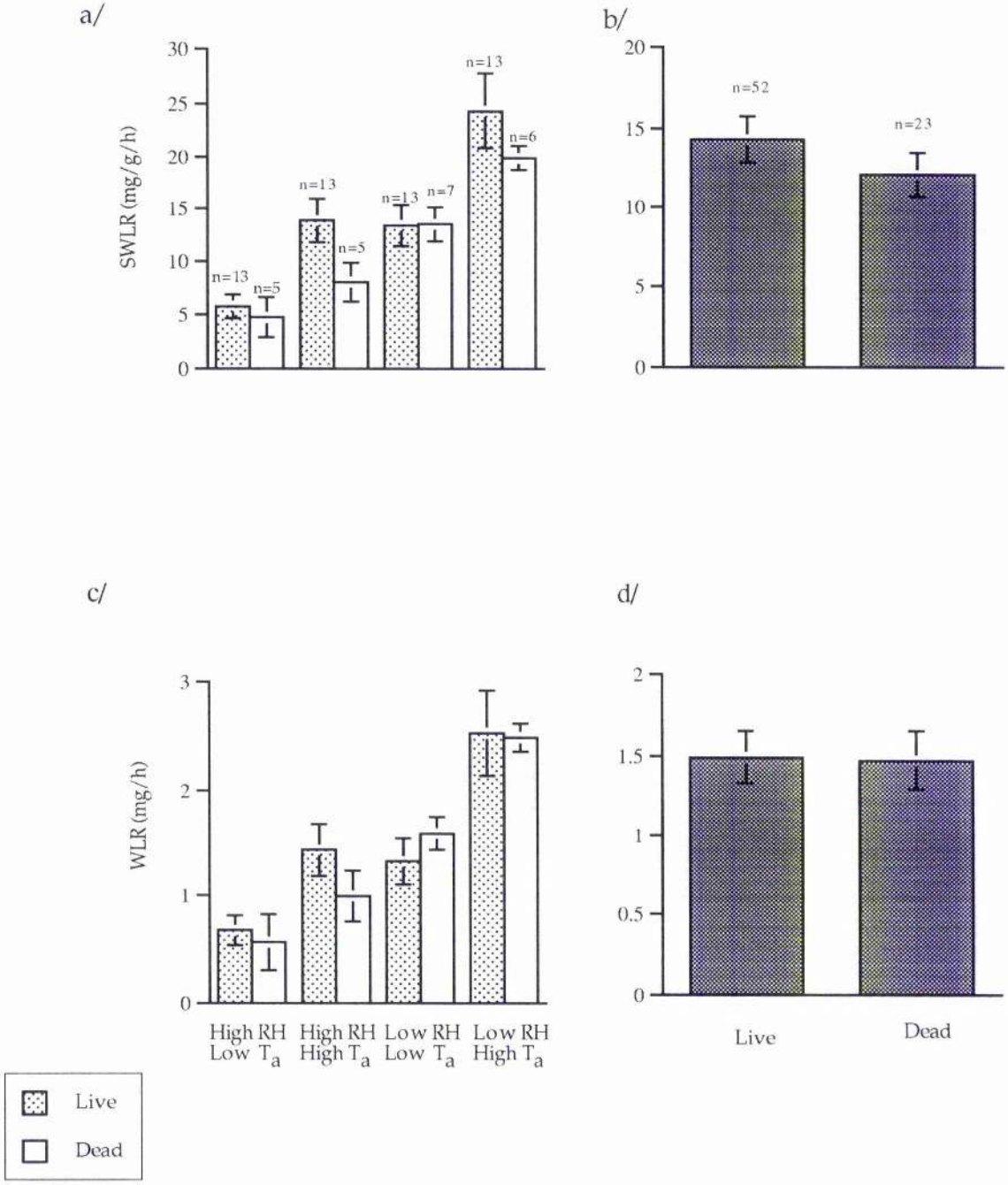


Fig. 3.5 Water loss in live and dead *E. tenax* in summer

a/ Specific rate of water loss vs conditions
b/ Specific rate of water loss vs live/dead state (four conditions grouped)
c/ Rate of water loss vs conditions
d/ Rate of water loss vs live/dead state (four conditions grouped)

b/ Winter

In winter, large live *E. tenax* (females) lose water faster than small ones (Table 3.18); there is no relationship between mass and specific water loss rate (Table 3.19). Figure 3.4d shows the relationship between mass and water loss rate (controlled for condition) (n=65, R^2=0.15, mass p=0.002). Condition is again a very significant factor (Figure 3.6a & c).

Table 3.18 Covariance analysis on the rate of water loss for mass and condition in live *E. tenax* in the winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	2.879	2.996	2.996	10.38	0.002
Condition	3	26.599	26.599	8.866	30.72	<0.001
Error	60	17.318	17.318	0.289		
Total	64	46.796				

Fitted mean for rate of water loss

	Mean (mg/h)	Stdev (mg/h)
High RH Low T_a	0.43	0.12
High RH High T_a	1.18	0.14
Low RH Low T_a	0.84	0.13
Low RH High T_a	2.15	0.14

Table 3.19 Covariance analysis on the specific rate of water loss for mass and condition in live *E. tenax* in the winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	17.47	22.19	22.19	1.11	0.297
Condition	3	1910.57	1910.57	636.86	31.79	<0.001
Error	60	1201.88	1201.88	20.03		
Total	64	3129.92				

Fitted mean for specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
High RH Low T_a	3.52	1.00
High RH High T_a	10.08	1.20
Low RH Low T_a	6.87	1.12
Low RH High T_a	18.03	1.16

There is no relationship between mass and water loss rate or specific water loss rate in dead *E. tenax* in winter (Tables 3.20 and 3.21). Water loss is very much affected by conditions of temperature and humidity (Figure 3.6a & c and Tables 3.20 and 3.21).

Table 3.20 Covariance analysis on the rate of water loss for mass and condition in dead *E. tenax* in the winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	1.590	1.952	1.952	2.90	0.097
Condition	3	35.524	35.524	11.841	17.56	<0.001
Error	36	24.274	24.274	0.674		
Total	40	61.387				

Fitted mean for rate of water loss

	Mean (mg/h)	Stdev (mg/h)
High RH Low T_a	0.74	0.26
High RH High T_a	1.31	0.27
Low RH Low T_a	1.41	0.25
Low RH High T_a	3.17	0.25

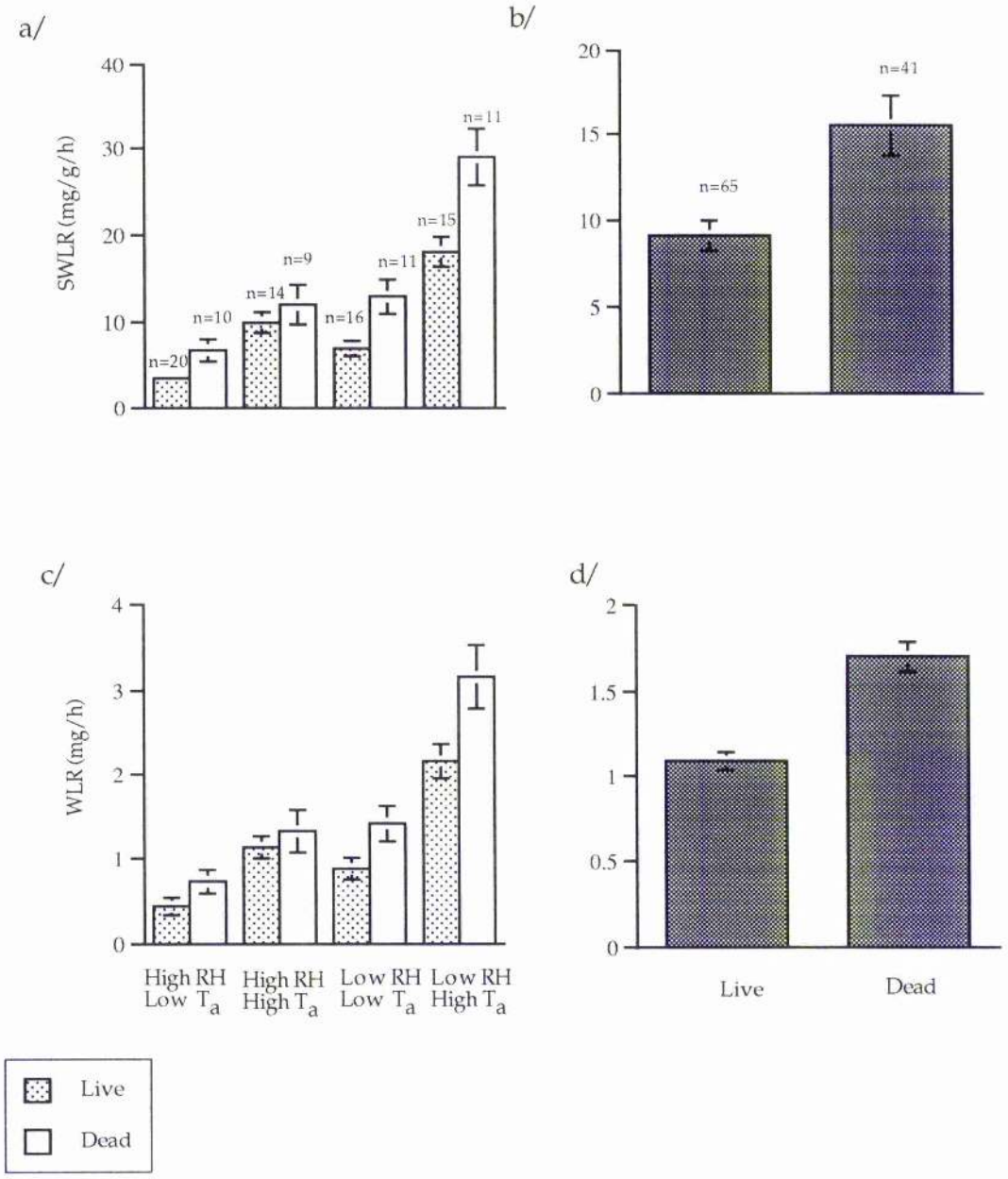


Fig. 3.6 Water loss in live and dead *E. tenax* in winter

a/ Specific rate of water loss vs conditions
b/ Specific rate of water loss vs live/dead state (four conditions grouped)
c/ Rate of water loss vs conditions
d/ Rate of water loss vs live/dead state (four conditions grouped)

Table 3.21 Covariance analysis on the specific rate of water loss for mass and condition in dead *E. tenax* in the winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.15	2.71	2.71	0.05	0.832
Condition	3	2961.38	2961.38	987.13	16.55	<0.001
Error	36	2147.82	2147.82	59.66		
Total	40	5109.35				

Fitted mean for specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
High RH Low T_a	6.78	2.44
High RH High T_a	11.94	2.58
Low RH Low T_a	12.89	2.33
Low RH High T_a	29.03	2.33

B/ *E. pertinax*

Covariance analyses for mass, condition and sex on water loss rate and specific water loss rate were carried out.

There is a positive relationship between mass and water loss rate in live flies (Figure 3.7a, Table 3.22) and a negative relationship between mass and specific water loss rate in dead flies (Figure 3.7b, Table 3.25). Thus, large live flies lose water faster than small ones. Small dead flies lose water faster per unit mass than large ones.

As for *E. tenax*, condition is a very significant factor both in live and dead flies (Tables 3.22, 3.23, 3.24 and 3.25). Figure 3.8a & c shows how water loss is affected by conditions of temperature and humidity in live and dead *E. pertinax*.

Sex is also a significant factor in live flies, with males losing water faster than females at all conditions except at low humidity and high temperature, the most desiccating condition (Figure 3.9 and Table 3.22 and 3.23). In dead flies, sex is not a significant factor, but there is a trend for males to lose water faster than females, especially at low relative humidity (Figure 3.10 and Tables 3.24 and 3.25).

Table 3.22 Covariance analysis on the rate of water loss for mass and condition in live *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.247	2.225	2.225	13.14	0.001
Sex	1	1.104	1.450	1.450	8.57	0.005
Condition	3	8.171	8.171	2.724	16.08	<0.001
Error	48	8.128	8.128	0.169		
Total	53	17.649				

Fitted mean for rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Females	0.76	0.08
Males	1.12	0.09
High RH Low T_a	0.44	0.10
High RH High T_a	0.95	0.10
Low RH Low T_a	0.81	0.13
Low RH High T_a	1.55	0.12

Table 3.23 Covariance analysis on the specific rate of water loss for mass condition and sex in live *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	303.63	0.79	0.79	0.03	0.859
Sex	1	160.50	223.49	223.49	8.97	0.004
Condition	3	1692.91	1692.91	564.30	22.64	<0.001
Error	48	1196.27	1196.27	24.92		
Total	53	3353.31				

Fitted mean for the standardised rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Females	10.71	0.98
Males	15.15	1.06
High RH Low T_a	6.28	1.26
High RH High T_a	11.98	1.25
Low RH Low T_a	11.13	1.58
Low RH High T_a	22.32	1.48

Table 3.24 Covariance analysis on rate of water loss for mass, condition and sex in dead *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	1.440	0.262	0.262	0.73	0.408
Sex	1	1.883	0.928	0.928	2.58	0.131
Condition	3	13.903	13.903	4.634	12.87	<0.001
Error	14	5.041	5.041	0.360		
Total	19	22.267				

Fitted mean for rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Females	1.09	0.19
Males	1.58	0.21
High RH Low T_a	0.37	0.28
High RH High T_a	0.71	0.28
Low RH Low T_a	1.55	0.28
Low RH High T_a	2.70	0.28

Table 3.25 Covariance analysis on the specific rate of water loss for mass, condition and sex in dead *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	39.91	598.31	598.31	10.95	0.005
Sex	1	434.17	226.39	226.39	4.14	0.061
Condition	3	1836.92	1836.92	612.31	11.20	0.001
Error	14	765.27	765.27	54.66		
Total	19	3076.27				

Fitted mean for the standardised rate of water loss

	Mean (mg/g/h)	Stdev
Females	12.12	2.35
Males	19.64	2.62
High RH Low T_a	3.86	3.49
High RH High T_a	9.78	3.45
Low RH Low T_a	18.57	3.40
Low RH High T_a	31.32	3.41

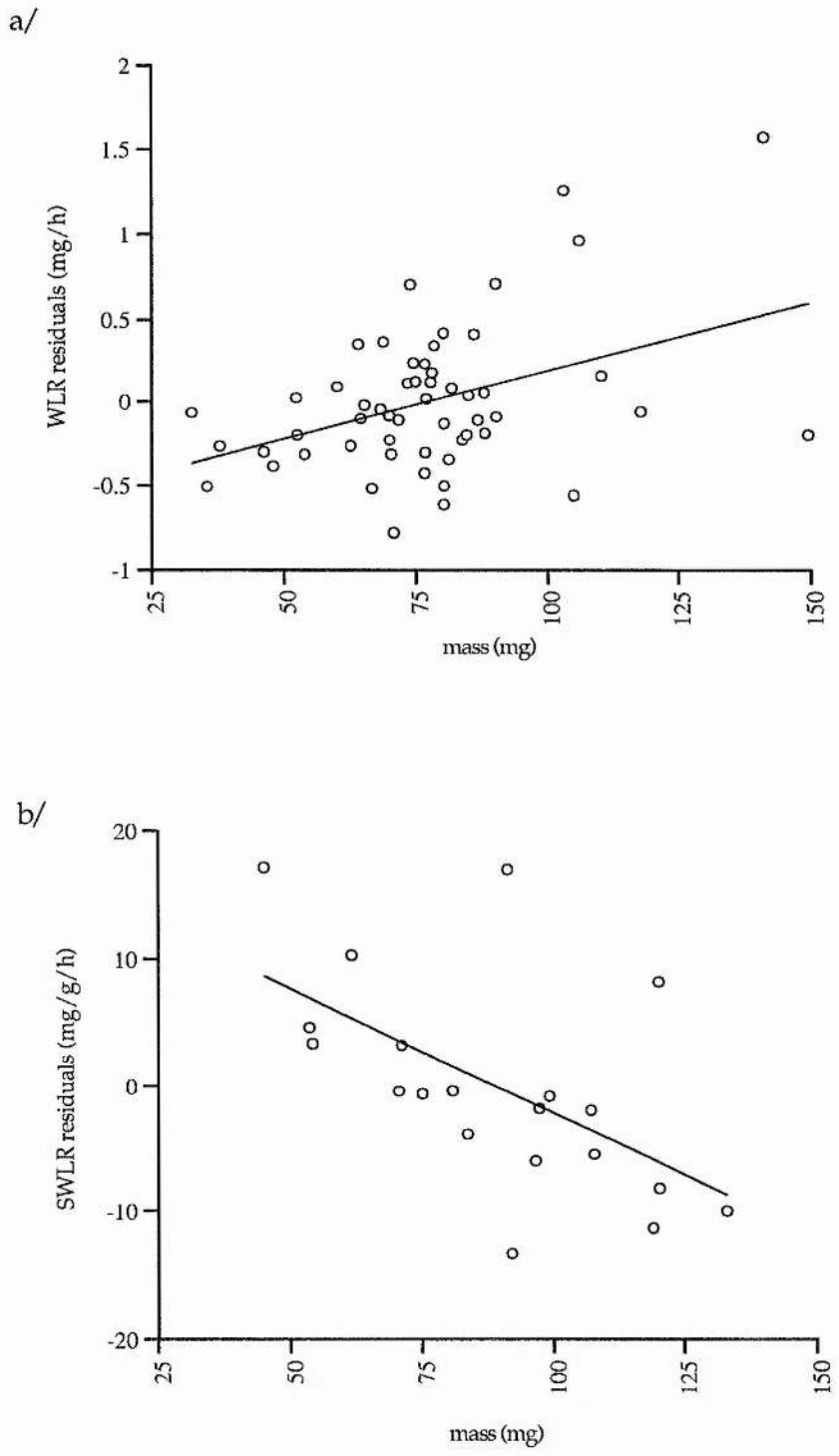


Fig. 3.7 Relationships between mass and rate of water loss and specific rate of water loss in *E. pertinax*. Conditions and sex have been controlled for, so the residuals of the glm analyses are plotted against mass.

a/ Live flies - rate of wate loss vs mass: $y = 0.008x - 0.641$, $r^2 = 0.17$
b/ Dead flies - specific rate of water loss vs mass: : $y = -0.197x + 17.553$, $r^2 = 0.34$

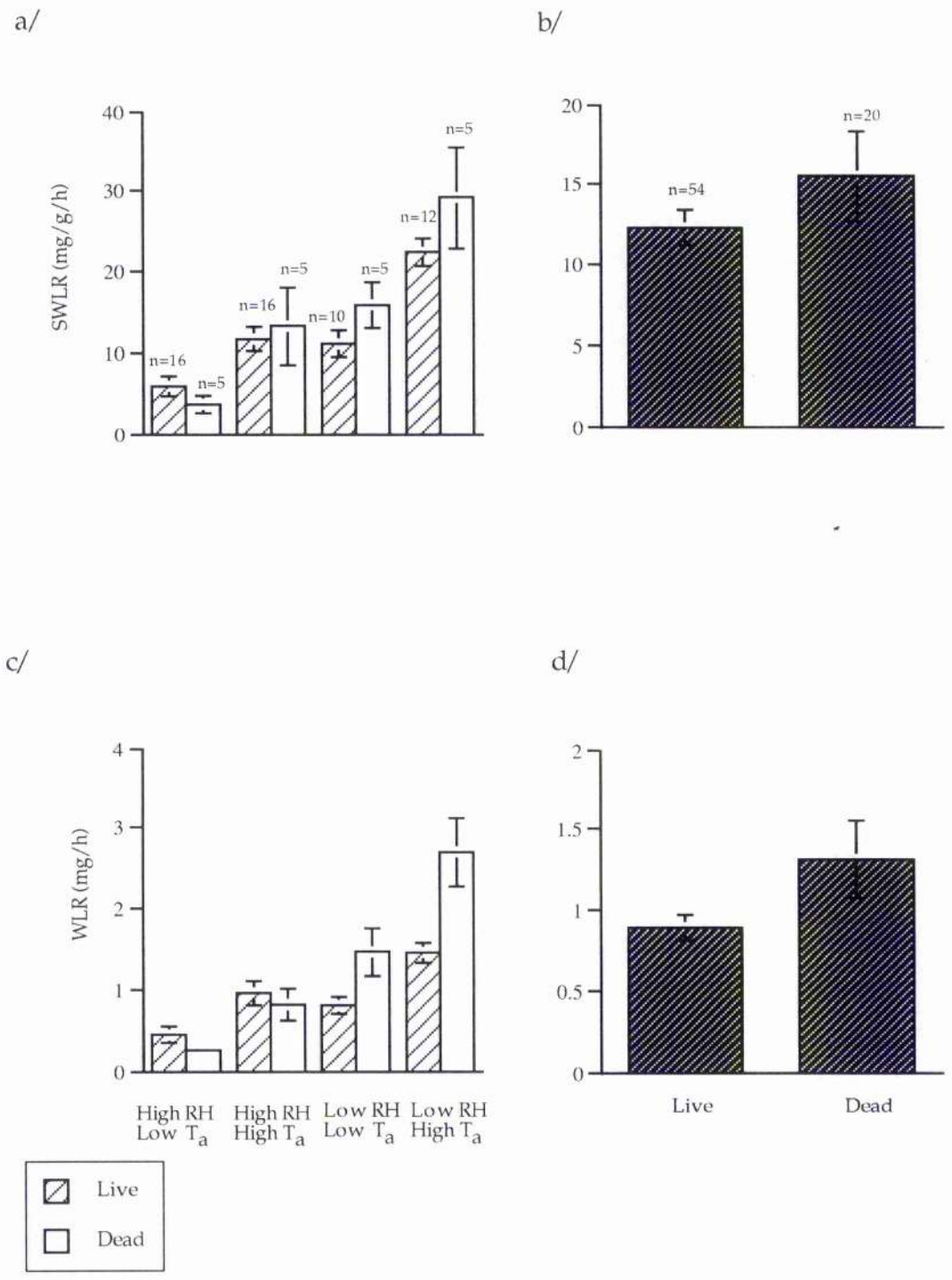


Fig. 3.8 Water loss in live and dead *E. pertinax* (summer)

a/ Specific rate of water loss vs conditions
b/ Specific rate of water loss live vs dead (all conditions grouped)
c/ Rate of water loss vs conditions
d/ Rate of water loss live vs dead (all conditions grouped)

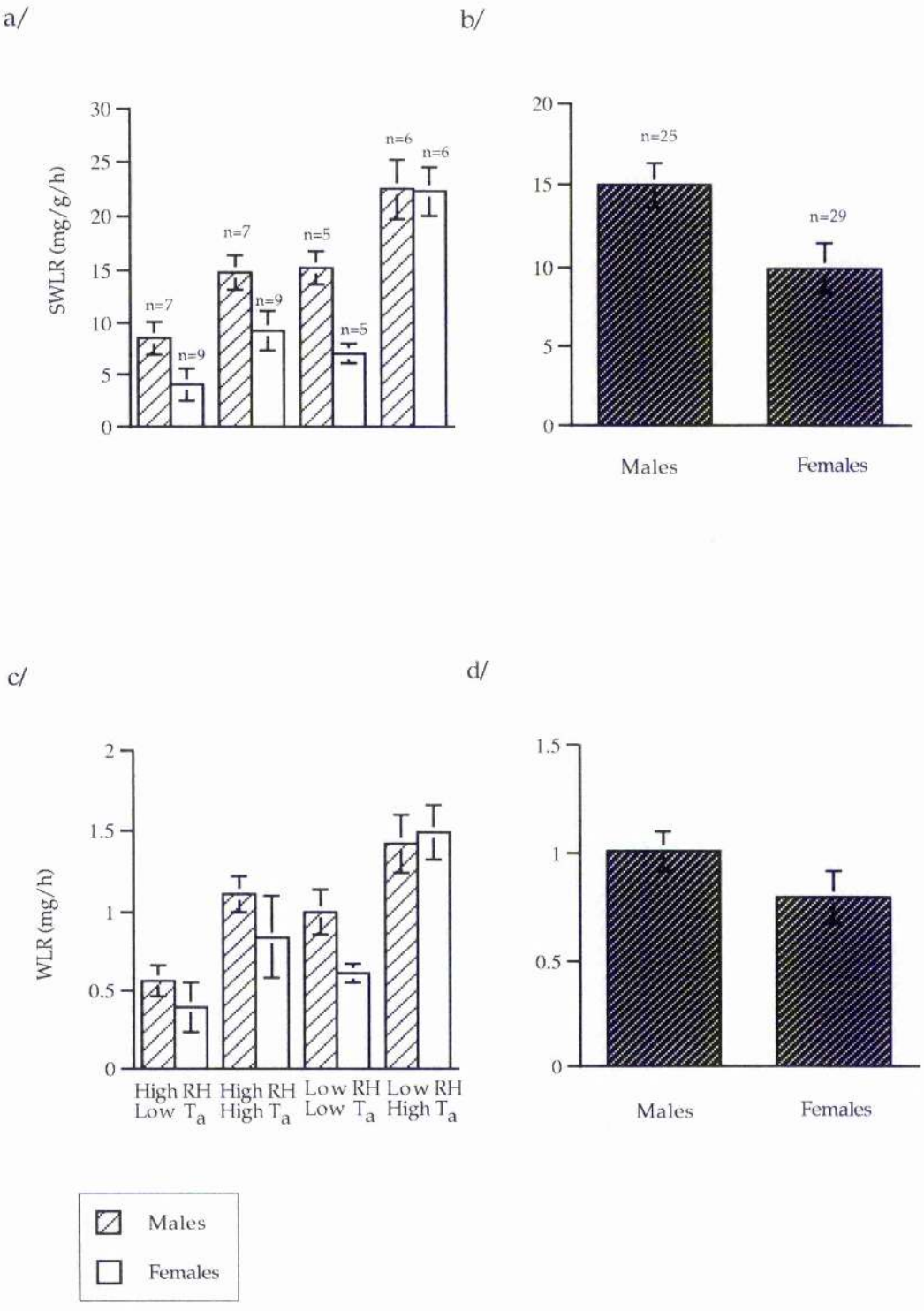


Fig. 3.9 Water loss in live female and male *E. pertinax*

a/ Specific rate of water loss vs conditions
b/ Specific rate of water loss vs males/females (four conditions grouped)
c/ Rate of water loss vs conditions
d/ Rate of water loss vs males/females (four conditions grouped)

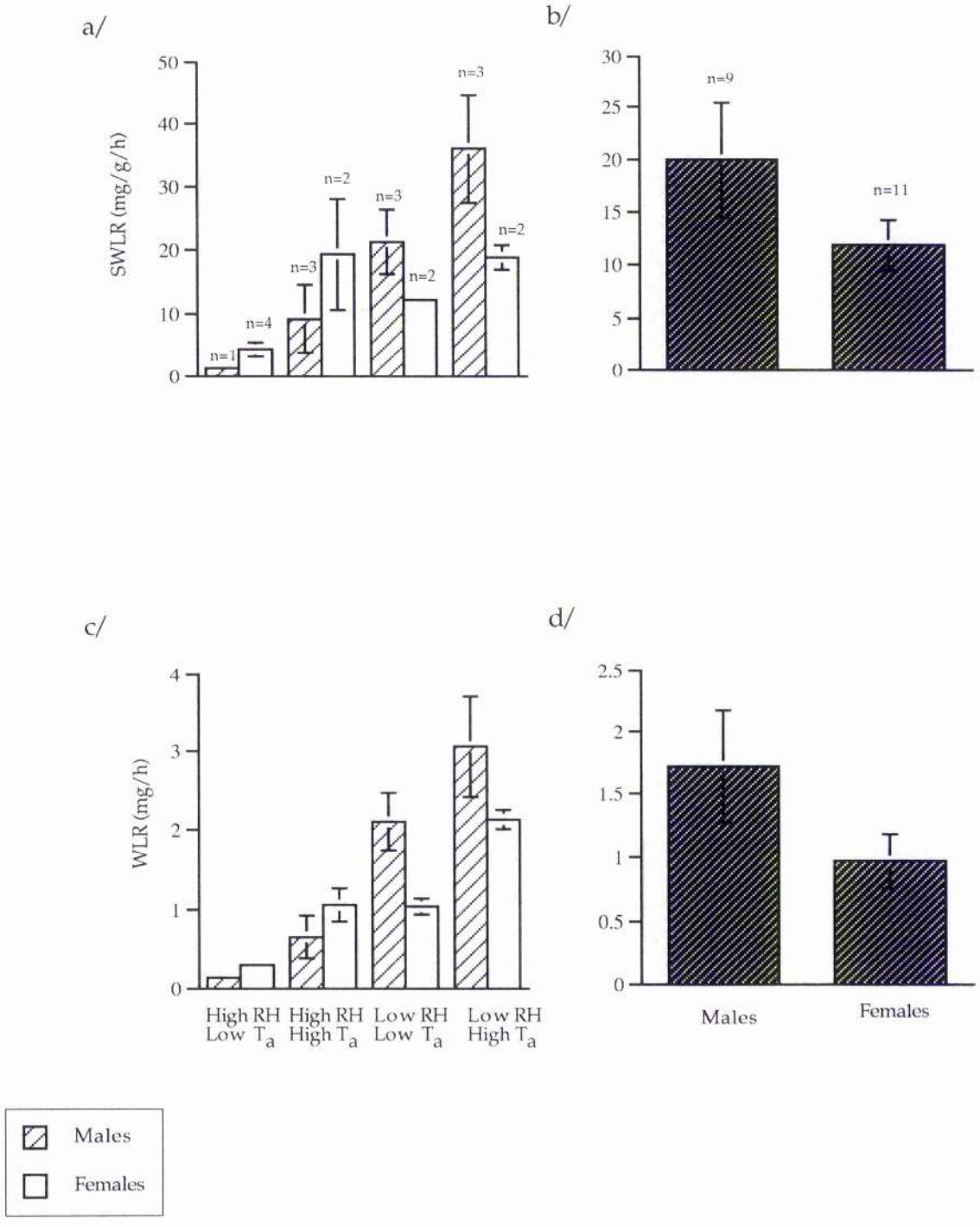


Fig. 3.10 Water loss in dead female and male *E. pertinax*

a/ Specific rate of water loss vs conditions
b/ Specific rate of water loss vs males/females (four conditions grouped)
c/ Rate of water loss vs conditions
d/ Rate of water loss vs males/females (four conditions grouped)

C/ Summary of section 3.3.3

• Relationships of rate of water loss and specific rate of water loss with mass were demonstrated in some, but not all, cases. For *E. tenax*, large live and dead males (summer) and live females (winter) lose water faster than small ones. This is true for live *E. pertinax* as well. In addition, large dead females (winter) *E. tenax* and large dead *E. pertinax* lose water more slowly per unit mass than small ones.

• For these eristalines, water loss is very much influenced by temperature and humidity conditions. As expected, they lose water at the slowest rate at high humidity and low temperature, and at the fastest rate at low humidity and high temperature, reflecting the variations in the desiccating effect of the air and the change in activity level, in live flies.

• Male and female *E. tenax* lose water at the same rate, but male *E. pertinax* lose water faster than females. This latter tendency, although not quite statistically significant, also exists in dead flies.

3.3.4 Live/ dead effect

A/ *E. tenax*

The covariance analyses on water loss rate and specific water loss rate for mass, condition and live/dead state reveals that, in summer, live and dead *E. tenax* lose water at the same rate. The rise in transpiration which accompanies the rise in temperature (constant humidity) or the decrease in relative humidity (constant temperature) is not greater in live than dead flies (Table 3.26 and 3.27 and Figure 3.5).

Table 3.26 Covariance analysis on the rate of water loss for mass, condition and live or dead state in *E. tenax* in summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	2.955	2.706	2.706	4.06	0.048
Condition	3	33.265	33.404	11.135	16.70	<0.001
Live/dead	1	0.368	0.368	0.368	0.55	0.460
Error	69	46.014	46.014	0.667		
Total	74	82.602				

Table 3.27 Covariance analysis on the rate of water loss for mass, condition and live or dead state in *E. tenax* in summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	106.80	93.08	93.08	1.77	0.187
Condition	3	2980.88	2910.45	970.15	18.48	<0.001
Live/dead	1	66.15	66.15	66.15	1.26	0.265
Error	69	3621.46	3621.46	52.48		
Total	74	6685.30				

Tables 3.28 and 3.29 and Figure 3.6 show that, once mass and condition have been controlled for, the live/dead state factor is highly significant in winter flies. Live *E. tenax* lose water more slowly than dead flies.

Table 3.28 Covariance analysis on the rate of water loss for mass, condition and live or dead state in *E. tenax* in winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	1.440	4.489	4.489	10.10	0.002
Condition	3	62.138	59.311	19.770	44.48	<0.001
Live/dead	1	9.338	9.338	9.338	21.01	<0.001
Error	100	44.443	44.443	0.444		
Total	105	117.360				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Live	1.11	0.08
Dead	1.74	0.11

Table 3.29 Covariance analysis on the specific rate of water loss for mass, condition and live or dead state in *E. tenax* in winter

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	26.3	15.8	15.8	0.43	0.512
Condition	3	4803.7	4576.0	1525.3	41.80	<0.001
Live/dead	1	795.8	795.8	795.8	21.81	<0.001
Error	100	3649.0	3649.0	36.5		
Total	105	9274.9				

Fitted mean for the specific rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Live	9.57	0.76
Dead	15.44	0.97

B/ ***E. pertinax***

Tables 3.30 and 3.31 reveal that once mass, sex, condition, and the interactions of the live/dead state with mass and sex have been controlled for, live/dead state is a significant factor: dead *E. pertinax* lose water faster than live flies. This is also shown in Figure 3.8.

Table 3.30 Covariance analysis on the rate of water loss for mass, condition, live or dead state, sex and the interactions between live or dead state and mass and between live or dead state and sex in *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	1.998	1.327	1.327	4.99	0.029
Sex	1	3.256	2.209	2.209	8.30	0.005
Condition	3	18.180	17.954	5.985	22.50	<0.001
Live/dead	1	0.993	1.158	1.158	4.35	0.041
Live/dead*mass	1	0.703	0.721	0.721	2.71	0.104
Live/dead*sex	1	0.030	0.030	0.030	0.11	0.738
Error	65	17.289	17.289	0.266		
Total	73	42.448				

Fitted mean for rate of water loss

	Mean (mg/h)	Stdev (mg/h)
Live	1.00	0.07
Dead	1.32	0.13

Table 3.31 Covariance analysis on the specific rate of water loss for mass, condition, live or dead state, sex and the interactions between live or dead state and mass and between live or dead state and sex in unrestrained *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	217.23	244.06	244.06	6.85	0.011
Sex	1	511.75	460.74	460.74	12.93	0.001
Condition	3	3108.05	3175.80	1058.60	29.72	<0.001
Live/dead	1	145.58	362.38	362.38	10.17	0.002
Live/dead*mass	1	255.80	268.04	268.04	7.52	0.008
Live/dead*sex	1	28.68	28.68	28.68	0.81	0.373
Error	65	2315.57	2315.57	35.62		
Total	73	6582.67				

Fitted mean for the standardised rate of water loss

	Mean (mg/g/h)	Stdev (mg/g/h)
Live	13.19	0.84
Dead	17.53	1.45

C/ Summary of section 3.3.4

Dead *E. tenax* from the winter generation and *E. pertinax* lose water faster than live flies. There is no difference in water loss rate between dead and live summer *E. tenax*.

3.3.5 Species difference?

In Chapter 4, where the passive rates of warming up and cooling down are discussed, it will be seen that *E. tenax* appears to be more affected by evaporative cooling than *E. pertinax*. Thus, the rate of water loss of the two species had to be compared. Only the actual rate of water loss is of interest here, as it indicates the extent of evaporative cooling. To avoid having to control for the season factor and to have more equilibrated data as far as sex is concerned for *E. tenax*, this comparison used summer flies. The covariance analysis for mass, condition and species on water loss rate shows that, in live flies, species is a significant factor. *E. tenax* loses water at a higher rate than *E. pertinax* (Table 3.32 and Figure 3.11a & b). This analysis is equivalent to the analysis for warming up and cooling down rates done in Chapter 4. There, no effect of sex was found. The sex factor not being a significant factor in the present analysis, it was left out. In dead flies, species is not a significant factor: there is no difference in the water loss rate of *E. tenax* and *E. pertinax* (Table 3.33). However, Figure 3.11c & d show that there is a trend towards *E. tenax* having higher losses than *E. pertinax*.

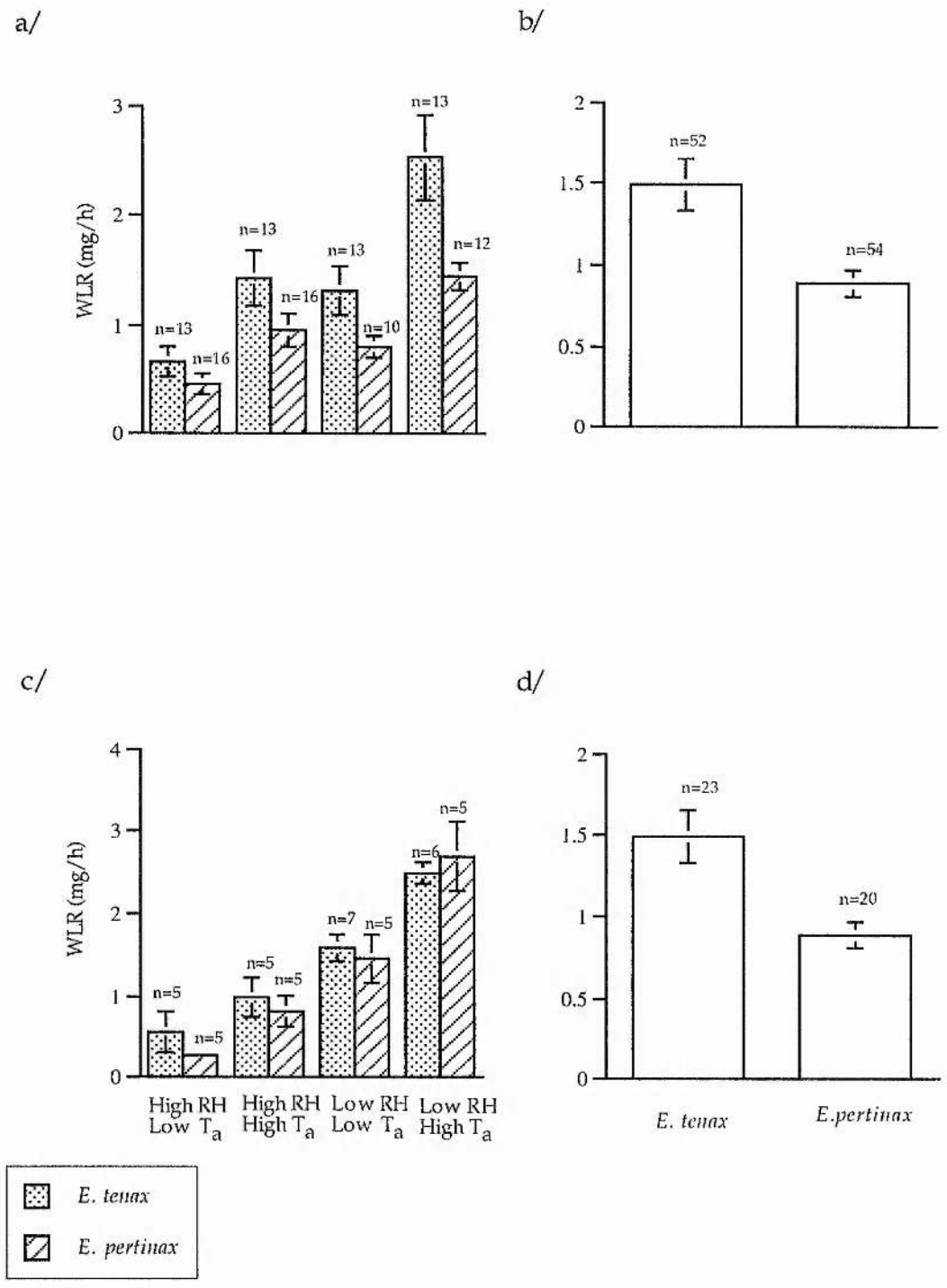


Fig. 3.11 Water loss in *E. tenax* and *E. pertinax* (summer flies)

a/ Water loss rate versus conditions in live flies
b/ Water loss rate versus species (all conditions grouped) in live flies
c/ Water loss rate versus conditions in dead flies
d/ Water loss rate versus species (all conditions grouped) in dead flies

Table 3.32 Covariance analysis on the rate of water loss for mass, condition and species in live E. *tenax* and *E. pertinax* in summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	7.522	4.077	4.077	7.67	0.007
Condition	3	31.293	29.526	9.839	18.52	<0.001
Species	1	2.115	2.115	2.115	3.98	0.049
Error	100	53.124	53.124	0.531		
Total	105	94.053				

Fitted mean for the rate of water loss

	Mean (mg/h)	Stdev (mg/h)
E. tenax	1.38	1.11
E. pertinax	1.05	1.11

Table 3.32 Covariance analysis on the rate of water loss for mass, condition and species in dead E. *tenax* and *E. pertinax* in summer

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	2.726	0.113	0.113	0.41	0.526
Condition	3	25.186	25.032	8.344	30.30	<0.001
Species	1	0.006	0.006	0.006	0.02	0.884
Error	37	10.190	10.190	0.275		
Total	42	38.109				

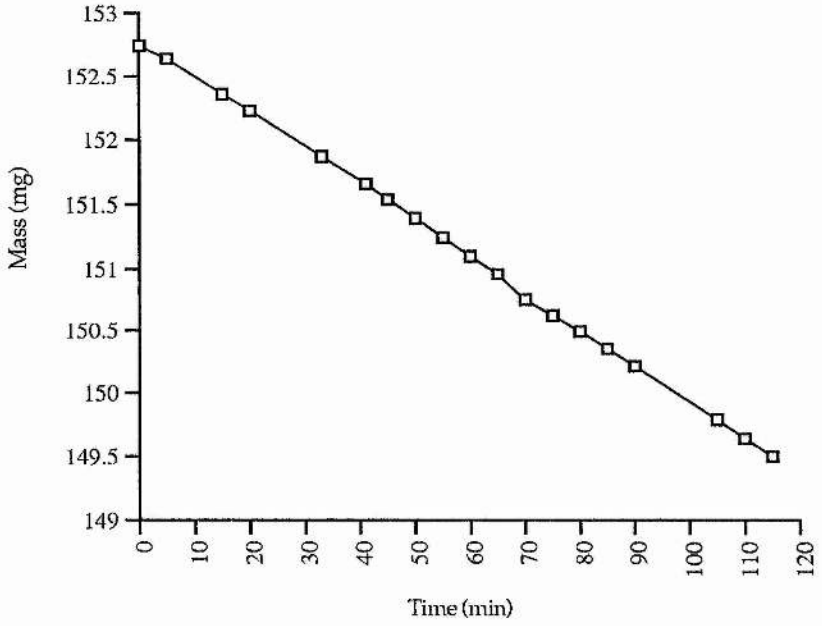
3.4 Discussion

3.4.1 Effect of restraining

Live and dead flies have lower water loss rates when restrained than when unrestrained, i.e. restraining the flies in a nylon net lowers the water loss rate. It is possible that a boundary layer which decreases the humidity gradient between the fly surface and air surrounding is created by using restraining material. In addition, water might condense on the net. Indeed, when restrained flies were tested at high humidity, mass began by increasing before decreasing. The mass of empty bags also began by increasing when placed at high humidity, suggesting that water condenses on the material. When a fly is placed in a bag, even at conditions of low humidity, a gradient of high humidity at the fly surface to low humidity some distance away is created. As the nylon mesh is very close to the fly, it is placed in conditions of high humidity and probably moisture condenses on it. The phenomena described above are probably responsible for decreasing the apparent rate of water loss of the restrained flies. This is possibly exacerbated by the fact that the air was unstirred in the test chamber.

This represents an additional problem of the gravimetric method. Insects have to be immobilised in some way if their mass is to be continuously recorded, but the immobilising material alters water loss rates. Even where air is flowing through the balance chamber, the netting material is likely to affect water loss rates. Regular weighing is not recommended either, because it disturbs and stresses the animals and often results in increased water losses, as was suggested by Machin et al (1991) working with cockroaches. Curves of mass against time (obtained during continuous mass loss recording), sometimes showed a rapid mass loss at the beginning, stabilising to a constant rate of mass loss within an hour (Figure 3.12). Such and effect can be attributed to the stress suffered by the insects and to dehydration of the cuticle. Loveridge (1968a) studying the locust *Locusta migratoria migratorioides* found that the decrease in rate of water loss only occurred at relative humidities between 0 and 50 % but not at 75 %. He explained this phenomenon by the loss of

a/



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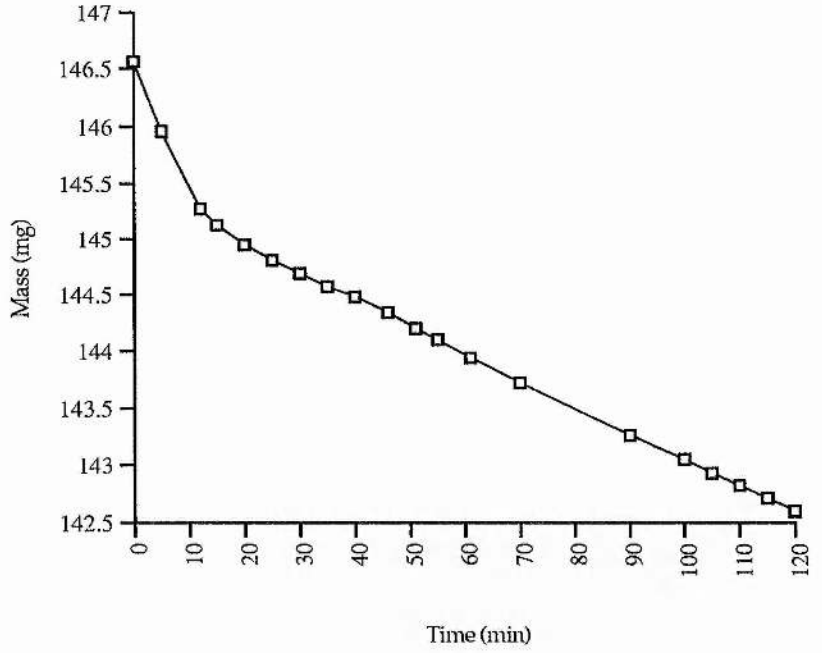


Fig. 3.12 Continuous measurement of mass loss (i.e. water loss) in two restrained *E. tenax* at low humidity high temperature

a/ Rate of water loss constant throughout the experiment
b/ Rate of water loss higher at the beginning

hydroscopic water during the initial period. Which of these affects the two eristalines studied is not clear. Comparing data obtained at low and high humidity is not of great help because of the problem of condensation that occurs at high humidity.

Also, restraining itself might stress the insect and cause increased water losses. In the present project, the problem was addressed by weighing the flies before and after their exposure to the test conditions during which they were unrestrained. Obviously, the longer the period between the weighings the better as it leaves time for the insect to recover from the stress of the first weighing. Here, the flies were left for 2 hours in the test pots. A few flies were left for 2 and then 4 hours (or for 4 and then 2 hours): their water loss rates were not systematically lower in the 4 hour experiments. Nevertheless, weighing every hour did seem to increase water losses, probably because of the extra disturbance. Therefore, the 2 hour period between the weighings seems appropriate for these experiments.

3.4.2 Effect of season on the water loss rate of *E. tenax*

The crucial finding is that the rate of water loss of female *E. tenax* is lower in winter than in summer. Overwintering flies seem to select very humid microhabitats (over 90% RH), probably to lower their water loss even further. *E. tenax* have been seen flying in winter (in December) in more southern parts of Britain (Kew gardens in London, Hasting 1988, and Cambridge, F. S. Gilbert, personal communication). In these locations they have access to flowering plants and can get energy and water. It would be interesting to investigate the overwintering patterns in these flies, for example to determine how often they leave their overwintering site and in which conditions, if they go back to the same overwintering site after coming out, and certainly if their water loss rate is different from that of flies in summer. In Scotland, *E. tenax* was not observed leaving or coming into the overwintering sites during winter, although some flies flew away when disturbed. Furthermore, no fly was seen foraging between December and February. Thus, it seems that during winter, Scottish *E. tenax* do not have access to drinking water, except when the overwintering environment becomes very humid and water drips from the walls. So *E. tenax* is likely to become dehydrated in winter. This is also

suggested by the observations that winter flies of the same thoracic width as summer flies are lighter and that haemolymph is so concentrated that it is impossible to withdraw. It would certainly be worthwhile to estimate water contents of summer and winter flies to confirm this claim.

Everything seems to point to the direction that, during winter, *E. tenax* is under water stress. In summer, water can easily be obtained in food (nectar), or by drinking, and through metabolic water production.

The results of the water balance experiments indicate that to cope with the water stress in winter, *E. tenax* lowers its water loss rate. It has been shown in several studies that dehydrated arthropods lose water at a lower rate than hydrated ones. Hadley and Quinlan (1993) demonstrated that dehydrated grasshoppers *Romalea guttata* have lower rates of water loss. They suggest that this decrease is not achieved by decreasing water losses associated with gas exchange (i. e. reducing frequency of the opening of the spiracles) but rather by lowering cuticular transpiration. However, Machin et al (1991) found that the cockroach *Periplaneta americana* reduces its ventilatory water losses in response to dehydration. This was also suggested by Loveridge (1968b) in his study of the locust *Locusta migratoria migratorioides*. Furthermore, Loveridge (1968a) showed that cuticular permeability is decreased at low external humidities in this locust. Which of these is responsible for the decrease in water loss rate in *E. tenax* in winter is unclear.

Cuticular permeability could be lowered in a number of ways. The quality and quantity of cuticular lipids could be altered to make the cuticle more waterproof. It is well described in the literature that cuticular lipid composition reflects the environment in which the arthropod lives (Hadley 1994). For example, low cuticular permeability is correlated with high amounts of cuticular lipids or greater quantities of a specific lipid class (e.g. hydrocarbons) (Hadley 1994). Hadley and Schultz (1987) demonstrated the higher hydrocarbon density in a tiger beetle species living in a xeric environment compared to a species living in a mesic environment. Furthermore, the percentage of long-chain hydrocarbons is greater in the epicuticle of summer than winter-active beetles and scorpions, so that water loss rate is lower (Hadley 1977, Toolson and Hadley 1979). These arthropods experience more desiccating conditions in summer, so lowering cuticular permeability in summer would seem a

good strategy. Also, acclimation of winter beetles (*Eleodes armata*) to an ambient temperature of 35 °C for five weeks, results in an increase of the amount of cuticular hydrocarbon (Hadley 1977). Thus, cuticular lipid composition and amount can be altered in face of a changing environment. In the case of *E. tenax*, water stress is more likely to be experienced by overwintering flies than active summer ones.

To determine how *E. tenax* lowers its water losses would require further experiments which were beyond the scope of this work. For example, the control of ventilatory losses could be investigated by relating continuously recorded mass losses to respiratory cycles (as described in Machin et al 1991) or by simultaneously measuring water loss and carbon dioxide production (as described in Hadley and Quinlan 1982). The cuticular lipid composition and density could also be investigated.

3.4.3 Water loss in live and dead eristalines

Water losses of dead arthropods tend to be greater than those of live ones, even when the spiracles and other openings are blocked (Hadley 1994). Here, no attempt was made to seal the spiracles of the flies, and as it was observed that these eristalines die with at least some of their spiracles open, it was expected that dead flies would have higher water losses than live ones.

This proved true for *E. tenax* in winter and for *E. pertinax*. However, no difference in water loss rate was found between live and dead *E. tenax* in summer. This suggests that *E. tenax* do not exert much control over their water losses in summer; it could also be linked to them being more active than in winter. Figure 3.5a & c shows that water losses tend to be higher in live flies than in dead flies at high temperature but not at low temperature. The level of activity was not checked, but this seems to support the contention that the flies were so active at high temperature that their water losses were very much increased and that not much control was exerted over these losses.

3.4.4 Effect of size

Cuticular water loss rate (expressed in mg H_2O h^{-1}) should be positively correlated with size as larger flies lose more water, in total, than small flies. If water loss rate is correlated with surface area, it should have

a negative relationship with mass when expressed as specific water loss rate (in mg H_2O g^{-1} h^{-1}) because big flies have a proportionally smaller surface area than small ones. However, it is not obvious if respiratory water loss is correlated with size. So, the combination of cuticular and respiratory water losses might not necessarily be correlated with mass. In this study, a positive relationship between water loss rate and mass was found in live male (summer), dead male, and live winter female *E. tenax* and in live *E. pertinax*. A negative relationship between specific water loss and mass was also demonstrated in dead summer and winter female *E. tenax* and in dead *E. pertinax*. However, this relationship could be an artefact. When water loss rate has no relationship with mass and individual values are divided by the corresponding mass, a negative relationship between water loss rate and mass is bound to appear. Therefore, the expected positive relationship between water loss rate and mass was found in some experiments but not in all of them, and this probably reflects the combination of respiratory and cuticular water losses. The relationship was most often found in live flies, which might be restricting their respiratory water loss. The spiracles of several dead flies were examined under the microscope: most flies die with at least some of the thoracic spiracles (but not necessarily all of them) open, but a few had apparently closed spiracles. This could explain the difficulties in showing a relationship between water loss rate and mass in dead flies.

3.4.5 Are males and females as good at controlling their water losses?

This seems to be the case for *E. tenax*: females and males lose water at the same rate. However, live male *E. pertinax* lose water faster than females, and there is a similar trend in dead flies. In live *E. pertinax*, males are more "leaky" than females in all conditions of temperature and humidity except at low humidity and high temperature, the most desiccating condition (Fig. 3.9a & b). In dead flies, males transpire more than females at low humidity but there does not seem to be a difference at high humidity (Fig. 3.10a & b). These results can be interpreted in the light of the water stress experienced by each sex. Male *E. pertinax* spend a great part of their time hovering (see Chapter 7), at least early in the summer. It was mentioned above that metabolic water production is high and

compensates for most of their evaporative water losses in hovering honeybees. This is likely to be true for *E. pertinax* as well. Indeed, extrusion of water from the anus by male *E. pertinax* was often noticed during observations of their hovering behaviour in St Andrews botanic garden. These males were thus probably in water excess. No data are available on the frequency of such extrusions. The great variability of hovering duration (Chapter 7) would make any investigation of this frequency difficult and unlikely to be of much use. When they feed, males take mostly nectar, except at the beginning of their life, when they need to mature their reproductive organs (Gilbert 1986). In contrast, females feed on both nectar and pollen throughout their lives and thus get less water than males through feeding. Therefore, it seems that of the two sexes, females are more water stressed than males and need to control their losses more carefully. This is supported by the present results: males are more "leaky" than females (live and dead). Only when conditions become too desiccating do males lower their water loss rate to a level comparable with females.

In the case of *E. tenax*, males do not hover much, and although they probably gain more water through feeding than females they might still be water stressed enough to need to control their water losses as well as females.

3.4.6 Water losses - effects of temperature and humidity, and comparison with other arthropods

A/ Cuticular permeability

In the present study, the flies were not restrained and became active at high temperature. Expressing the results as cuticular permeability is justified in resting animals and was done for species comparison purposes. Water losses were also expressed as water loss rate and specific water loss rate to compare the four conditions of humidity and temperature and to compare with published data where respiratory losses in relation to total water losses were investigated (these are expressed as the amount of water lost pert unit time).

Table 3.34 shows the cuticular permeability of *E. tenax* and *E. pertinax* (at 7 °C and 46% RH) and of other arthropods. Cuticular permeability was also calculated for 96% RH, but very high values were obtained (about 5

times higher than those estimated at 46% RH). These strongly suggest a problem with the method. Most probably, the high humidity environment was disturbed when the flies were introduced in the pots and took some time to be restored. During that time, the flies lost more water than expected. Therefore, when the effect of environmental condition comes to be discussed below, it should be remembered that the water losses at high relative humidity are probably substantial overestimates.

The data presented in Table 3.34 have been collected in a number of ways, all of which can affect the measured permeability. Perhaps the most realistic records are the ones by Nicolson et al (1984) in the tenebrionid beetle *Onymacris plana* and by Hadley et al (1989) in the cicada *Diceroprocta apache,* as they measured direct cuticular transpiration *in vivo* using isotopes. Others include ventilatory water loss to some extent, which can be exacerbated by handling for intermittent weighing or by the stress caused by restraining. Indeed, the cuticular transpiration recorded by Nicolson et al (1984) are between two and four times lower than those recorded by others using gravimetric methods. In addition, as pointed out above, techniques which use the amount of water in the air leaving the experimental chamber to estimate water losses do not take metabolic water production into account and record the gross amount of water lost. They are actually better than gravimetric methods to measure cuticular transpiration but do not tell as much about water balance and the net amount of water lost. However, assuming that the animals were resting, metabolic water production should not introduce an important error.

Therefore, it should be kept in mind that most of the data listed in Table 3.34 are only approximate values. The values obtained in the present study compare well with those for other arthropods living in mesic environments which range from 7.7 to 76 $\mu g\ cm^{-2}\ h^{-1}\ Torr^{-1}$. They are actually in the upper part of this range. Compared with the only two published records for flies of mesic environments (*Glossina palpalis* with 12 $\mu g\ cm^{-2}\ h^{-1}\ Torr^{-1}$ and *Calliphora erythrocephala* with 51 $\mu g\ cm^{-2}\ h^{-1}\ Torr^{1}$), *E. tenax* and *E. pertinax* are again in the upper range for cuticular permeability. Therefore, these two eristalines are rather "leaky" insects, and this suggests that they normally have access to enough water to compensate for their losses.

B/ Effect of humidity and temperature conditions and activity

It is very clear from the present results that humidity and temperature conditions have a large influence on these eristalines' water losses. It should however be remembered that the estimations of water loss rate at high humidity are very probably overestimates due to the technical problem mentioned above. Figures 3.5, 3.6 and 3.8 show that an increase in temperature at a constant humidity or a reduction of humidity at a constant temperature both lead to a rise in transpiration. This purely reflects the increased desiccating effect of air in these situations in dead flies, and such an effect is well documented in the literature (Edney 1977 and Hadley 1994). In live flies, the increase in respiratory water loss with increased temperature and activity also plays a part in the rise of water loss. It is not possible with the data expressed as water loss to distinguish between these effects. At best, the contribution of activity could be reflected in a greater rise in live than in dead flies. This does not seem to be the case here, what is possibly explained by the fact that the flies die with some of their spiracles open, thus including a "respiratory" component in the total water loss. Live flies also probably control their water losses. In addition, the present data show the *net* water loss experienced by these flies. Metabolic water produced when the animals are active somewhat compensates for the increased loss. With these data, not much more can be said on the effect of activity. However, activity is known to increase gross water losses. This has been demonstrated in honeybees by Louw and Hadley (1985) and in carpenter bees by Nicolson and Louw (1982) (see Table 3.35). Strangely enough, active bees are more likely to maintain their water balance than resting ones in the same environmental conditions due to the metabolic water produced. It is possible that the same applies to these eristalines.

Overwintering flies are probably losing water more slowly than was measured here. Selection of a suitably humid microhabitat and the low temperatures experienced in winter help reduce water losses but cannot completely eliminate them. Over the few months that overwintering lasts, *E. tenax* gets dehydrated, but nevertheless survives. Two flies were kept in the laboratory in plastic boxes, with 97 ± 2 % RH inside and at an ambient temperature of 5 ± 1 °C, from mid-October (no food available). Both survived until mid-February. The mass of one decreased from 163 mg to

112 mg (it lost 31% of its initial mass), while that of the other went from 124 mg to 63 mg (a reduction of 49%). The latter's mass increased to 90 mg when water was made available. Thus, overwintering *E. tenax* do become dehydrated but can withstand that state, and selection of suitable overwintering sites and physiological control help in limiting water losses.

Certainly, these syrphids would experience considerable water losses on hot and dry days when not active. It is however very difficult to compare their losses with those of other arthropods. To be comparable, data must have been obtained for net water losses in similar conditions of temperature and humidity, but most of the data from other authors were collected in dry air at 30 °C, being much more desiccating conditions. Nevertheless, a broad comparison with the species listed in Table 3.35 confirms that these two eristalines are not very efficient at keeping water. For example, the cricket *Acheta domesticus* loses 19.5 mg H_2O g^{-1} h^{-1} when active (Hadley and Quinlan 1982) whereas *E. tenax* loses between 18.05 and 24.28 mg H_2O g^{-1} h^{-1} and *E. pertinax* 22.38 mg H_2O g^{-1} h^{-1} when active, but in much less desiccating conditions. Likewise the locust *Locusta migratoria migratorioides* loses a maximum of 5.3 mg H_2O g^{-1} h^{-1} at rest in very desiccating conditions (Loveridge 1968a) when *E. tenax* loses between 6.96 and 13.36 mg H_2O g^{-1} h^{-1} and *E. pertinax* 11.13 mg H_2O g^{-1} h^{-1} (at 7 °C - thus resting - and 46% RH). Again, tenebrionids lose between 0.2 and 0.4 mg H_2O g^{-1} h^{-1} at 30 °C, 75% RH (in flowing air) whereas *E. tenax* loses between 9.93 and 13.85 mg H_2O g^{-1} h^{-1} and *E. pertinax* 11.68 mg H_2O g^{-1} h^{-1} at 20 °C, 96% RH. Thus, overall, these two syrphids are quite "leaky".

It is not possible to deduce anything from the present data as far as physiological control is concerned (except for the two generations of *E. tenax* as mentioned above). As already explained, using dead flies as a control is not of a great help. One way around this problem would be to have resting flies exposed to different conditions and compare their rate of water loss expressed in units of cuticular permeability. The desiccating effect of the environment would thus be controlled for, and any discrepancy between the conditions could be attributed to water loss control. However, this would only be valid if respiratory losses do not become an important component of the total water loss in some conditions, such as increased ambient temperature. Direct measurement of cuticular permeability (*in vivo* with isotopes) could eliminate this problem.

Control of respiratory water losses could be investigated by the methods described above and in particular the simultaneous recording of water loss and CO_2 emission. By separating the cuticular and spiracular components of water loss it would be easier to investigate physiological regulation.

3.4.7 Species difference?

Experiments on the rate of warming up and cooling down suggest that *E. tenax* is more affected by evaporative cooling than *E. pertinax* (see Chapter 4). This was shown to be true for live flies (Table 3.32, Figure 3.11a). Although the same trend exists for dead flies, the difference is not significant (Table 3.33, Figure 3.11b). Therefore, it seems that, in summer, *E. tenax* is not as good at controlling its water losses as *E. pertinax*. Being in general larger, *E. tenax* might not be as stressed for water balance as *E. pertinax*, or might be better at getting water through feeding. If water balance is not a problem for these eristalines in summer, it might be advantageous for *E. tenax* to be more "leaky" in relation to controlling its thermal balance, as it is likely to be more prone to overheating due to its larger size. The water lost helps cooling by evaporative cooling. Certainly, when water stressed, *E. tenax* is able to control its water losses, as seen for overwintering flies.

This chapter has investigated water losses in *E. tenax* and *E. pertinax* in conditions comparable to those encountered in the field, using a simple gravimetric technique. It was demonstrated that restraining insects to obtain continuous mass loss records interferes with water losses, probably by creating a humid boundary layer. Also, at high humidity, water tends to condense on the restraining material.

Temperature increase and humidity drop lead to higher water losses. Air becomes more desiccating at high temperature and low humidity, and in addition flies become more active at high temperature, thus losing more water through respiration.

With records from unrestrained flies, it is clear that *E. tenax* do not control their water losses much in summer compared to *E. pertinax*. However, a mixture of site selection and physiological control of water

losses help females to limit the amount of water they lose during overwintering. They nevertheless become dehydrated, but seem able to survive quite high levels of dehydration (up to 49% measured).

Male *E. pertinax* tend to lose more water than females. They only regulate their rate of water loss when conditions are very desiccating, but then they are as efficient as females. It is suggested that this stems from the fact that males are generally less water stressed than females because they feed on more nectar and they spend more time flying (hovering) thus producing more metabolic water.

For *E. tenax*, both sexes have similar water loss rates. Although males possibly get more water than females from feeding, they probably do not produce more metabolic water, and both are therefore likely to experience a similar water stress.

These eristalines have comparable water loss rates to other arthropods of similar size. The values calculated here tend to be in the higher part of the range for mesic animals, suggesting that these flies are relatively "leaky".

Table 3.34 Insect and some arachnid transpiration rates (measured at 30 °C and dry air, <5 % RH unless stated otherwise). Modified from Hadley (1994) and Edney (1977).

Taxon	Habitat	Permeability ($\mu g\ cm^{-2}\ h^{-1}\ Torr^{-1}$)	Remarks	Reference
Insecta				
Orthoptera				
Trimerotropis suffosa	Mesic	24.2		Massion (1983)
T. pallidipennis	Xeric	15.2		Massion (1983)
Aeropedellus clavatus	Mesic	67.7	Alpine tundra	Hadley & Massion (1985)
Locusta migratoria	Xeric	22.0	Dead	Loveridge (1968a)
Arphia spp	Mesic	10.3-15.3	22 °C	Forlow & MacMahon (1988)
Acheta domesticus	Mesic	59.0	0-3 h	Hadley et al (1986)
		23.0	3-6 h	
		16.0	6-12h	
Romalea guttata	Mesic	11	Whole, flow-through	Calulated from Hadley &Quinlan (1993)
Dictyoptera				
Arenivaga spp	Xeric	12.1-80.6		Cohen & Cohen (1981) and Edney &McFarlane (1974)
Periplaneta spp	Mesic	55-57		Mead-Briggs (1956) and Appel et al (1986)
Diploptera punctata	Mesic	20.9	Gravimetric, still air, dead	Appel (1991)
Pycnoscelus surinamensis	Mesic	38.7		Appel (1991)
Homoptera				
Diceroprocta apache	Xeric	110.0 (♂)	Whole animal, 45 °C	Toolson (1987)
		85.0 (♀)		
		244.0	27.2 °C; *in vivo* (cut. transp.)	Hadley et al (1989)
		sweating		

Table 3.34 continued

Taxon	Habitat	Permeability ($\mu g\ cm^{-2}\ h^{-1}\ Torr^{-1}$)	Remarks	Reference
Hemiptera				
Rhodnius prolixus	Xeric	12.0		Holdgate & Seal (1956)
Geocoris punctipes	Xeric	14	27 °C	Cohen (1982)
Lygus hesperus	Xeric	13	27 °C	Cohen (1982)
Coleoptera				
Arenivaga apacha	Me/Xe	80.6	Whole, grav., still air, < 5%, 20-30 °C	Cohen & Cohen (1981)
Arenivaga investigata	Xeric	30.0		
Centrioptera muricata	Xeric	6.3	Whole, gravimetric	Ahearn (1970)
Cryptoglossa verrucosa	Xeric	8.4		Ahearn & Hadley (1969)
Eleodes armata	Xeric	17.2		
Onymacris plana	Xeric	0.75	*In vivo* isotop.	Nicolson et al (1984)
		3.1	Wh., flow. air	Hadley and Louw (1980)
		1.53	Wh., still air	Edney (1971)
Lepidochora discoidalis	Xeric	3.1		Hadley and Louw (1980)
Cysteodemus armatus	Xeric	22		Cohen and Pinto 1977
Cicindela spp	Mesic	32.0-49.0	Gravim. restr.	Hadley and Schultz (1987)
Cicindela obsoleta	Xeric	24.0	Gravim. restr.	Hadley and Schultz (1987)
Cicindela longilabris	Mesic	23.9		Schultz et al (1992)
Tenebrio molitor	Xeric	1	Pupae	Holdgate and Seal (1956)
Liparocephalus cordicollis	Hygric	175		Topps and Ring (1988)
Rhynchophorus cruentatus	Mesic	39.5		Weissling & Giblin-Davis (1993)

Table 3.34 continued

Taxon	Habitat	Permeability ($\mu g\ cm^{-2}\ h^{-1}$ Torr $^{-1}$)	Remarks	Reference
Diptera				
Glossina morsitans	Xeric	0.3	Pupae	Bursell (1958)
	Me/Xe	8		Bursell (1957)
Glossina palpalis	Mesic	12		Mead-Briggs (1956)
Bibio sp	Hygric	76		Wigglesworth (1945)
Calliphora erythrocephala	Mesic	51		Mead-Briggs (1956)
Eristalis tenax	**Mesic**	**42 (wint.)**	**Whole, unrestrained, gravim.- 7 °C, 46% RH**	**This thesis**
		68 (sum.)		
Eristalis pertinax	**Mesic**	**51**		
Hymenoptera				
Anaphes ovijentatus	Xeric	3.6 (♂)	Parasitic wasp	Jackson & Cohen (1984)
		8.0 (♀)		
Apis mellifera	Mesic	21.5	Wh.,resting	Louw & Hadley (1985)
Pogonomyrmex rugosus	Xeric	11.6	25 °C	Lighton & Feener (1989)
		26.1 (workers)		R.A. Johnson (unpublished)
		9.3 (♂)		
		9.4 (♀)		

Table 3.34 continued

Taxon	Habitat	Permeability ($\mu g\ cm^{-2}\ h^{-1}\ Torr^{-1}$)	Remarks	Reference
Messor pergandei	Xeric	18.5 (workers) 13.6 (♂) 9.2 (♀)		R.A. Johnson (unpublished)
Coptotermes formosanus	Mesic	37.5 (workers)	Grav., interm. unrestr.	Sponsler & Appel (1990) Sponsler & Appel (1990)
Reticulitermes flavipes	Mesic	27.8 (workers)	Fire ant	
Solenopis invicta	Mesic	25.2		Appel et al.(1991)
Arachnida				
Scorpiones				
Pandinus imperator	Mesic	76		Cloudsley-Thompson (1959)
Eusorpius germanus	Mesic	31.4		Cloudsley-Thompson (1956)
Uroctonu apacheanus	Mesic	7.70		Toolson & Hadley (1977)

Table 3.35 Insect and some arachnid water loss rates.

Taxon	Water loss rate (mg g^{-1} h^{-1})	Remarks	Reference
Hoverflies		Whole animals, free to move; weighed before and after	**This thesis**
Eristalis tenax (winter)	**3.53**	**7°C, 96% RH**	
	9.93	**20°C, 96% RH**	
	6.96	**7°C, 46% RH**	
	18.05	**20°C, 46% RH**	
Eristalis tenax (summer)	**5.78**	**7°C, 96% RH**	
	13.85	**20°C, 96% RH**	
	13.36	**7°C, 46% RH**	
	24.28	**20°C, 46% RH**	
Eristalis pertinax	**5.97**	**7°C, 96% RH**	
	11.68	**20°C, 96% RH**	
	11.13	**7°C, 46% RH**	
	22.38	**20°C, 46% RH**	
Honeybees *Apis mellifera*		30 °C, < 5% RH Whole animals; flow-through respirometer	Louw & Hadley (1985)
	18.95	Rest (met. water: 2.39)	
	25.76	Moderate activity	
	38.73	High activity	
	79.72	Hovering flight (met.water: 74.36 mg g^{-1} h^{-1})	
Carpenter bees *Xylocopa capitata*		25 °C; Whole animals; flow-through respirometer	Nicolson & Louw (1982)
	17.1	Tethered flight	
	26.6	Free flight Met. water production > EWL at T_a < 27 °C but < EWL at T_a > 27°C	

Table 3.35 continued

Taxon	Water loss rate (mg g^{-1} h^{-1})	Remarks	Reference
Cockroaches			
		Gravimetric, flowing air	Cohen & Cohen 1981
Arenivaga apacha	8.7	22 °C, 5% RH	
	52.4	32 °C, 5% RH	
A. investigata	7.9	22 °C, 5% RH	
	15.6	32 °C, 5% RH	
Crickets		40 °C, dry air	Hadley and Quinlan (1982)
Acheta domesticus		Whole animals; flow-through respirometer	
	15.3	Rest	
	19.5	Active	
Locusts		Whole; gravimetric (before and after)	
Locusta migratoris migratoriodes	3.2	Dead	Loveridge (1968a)
	5.3	Hydrated	Loveridge (1968b)
	3.2	Dehydrated	
Grasshoppers		Dry air (<0.5% RH)	Hadley & Quinlan (1993)
Romeala guttata		Whole animals resting; flow-through respirometer	
	1.7	15 °C; hydrated	
	3.3	25 °C ; hydrated	
	4.9	30 °C ; hydrated	
	1.2	15°C ; dehydrated	
	2.0	25 °C ; dehydrated	
Tenebrionid beetles		22.0-25.3 °C, 27.5-46.0% RH; resting	
Onymacris plana	0.44	Gravimetric	Nicolson et al (1984)
	0.24	Ventilated capsule and tritiated water	

Table 3.35 contimued

Taxon	Water loss rate (mg g^{-1} h^{-1})	Remarks	Reference
Eleodes armata		Whole animal, gravimetric in flowing air; 30 °C; after 5h	Estimated from Ahearn (1970)
	2.4	0% RH	
	1.6	40% RH	
	0.4	75% RH	
Cryptoglossa verricosa	0.8	0% RH	
	0.4	40% RH	
	0.2	75% RH	
Ants		25°C, dry air; gravimetric	Lighton (1992)
Campomotus vicinus	7.17		
Cataglyphis bicolor	7.28		
Tarantula spiders			
Eurypelma helluo	6.0		Herreid (1969)
E. californicum	5.0		
Scorpions			
African scorpions		33°C, 0% RH	
Leiurus quinquestriciius	0.30		Cloudsley-Thompson (1956)
Androctonus australis	0.32		Cloudsley-Thompson (1961)
Sonoran scorpions			
Hadrurus hirsutus	0.42		Cloudsley-Thompson (1967)

Chapter 4 - Thermal balance: passive heat exchange

4.1 Introduction

As was mentioned in Chapter 1, the thermal biology of an insect depends very much on the size, the shape, the reflectance and the conductance of the cuticle of the animal. It is therefore important to determine how an insect is affected by passive body temperature changes before investigating the process of thermoregulation. Cooling and warming constants (k_c and k_w) reflect the speed at which an individual cools down or warms up and provide a good indication of how rapidly the body temperature of an animal changes in response to temperature changes in the environment. They also allow comparison with other animals and can be used to help explain activity patterns in the field.

Using live flies for estimating passive warming and cooling rates includes the risk that the rates are not passive (endothermy could increase the warming constant for example), but it can, by comparison with the rates of dead animals, reveal some kinds of physiological control of thermal exchanges. It is also a closer reflection of the real situation in the field. Therefore, both dead and live flies were used in this study.

In such investigations, the animal is placed in an environment where air temperature is different from the animal body's, and the change over time of the insect temperature is recorded. The Newtonian equation

$$dT_b/dt = k\,(T_b-T_a)$$

specifies the body temperature change per unit time per unit temperature difference between an animal and its surroundings (Bartholomew 1981). T_b is the body temperature (in °C), T_a the ambient temperature (in °C), and k the cooling or warming constant (in °C min^{-1} $°C^{-1}$). The data are conveniently analysed by plotting $\ln(T_b-T_a)$ against time, which gives a straight line if the temperature of the animal is changing with an

exponential rate. The constant k corresponds to the gradient of the line of this plot.

Most previous studies (e.g. Bartholomew 1981, Bartholomew and Epting 1975) have taken **ambient temperature** as the baseline to calculate the difference between "external" temperature and body temperature. However, Bakken (1976) warns of the dangers of doing so. The body temperature at which an animal equilibrates is not necessarily equal to ambient temperature. It can equilibrate at a higher temperature because of metabolic heat production, or at a lower temperature because of evaporative cooling. In this study, for example, the flies did not always equilibrate their thorax temperature at the same level as ambient temperature, but usually at a lower temperature (up to about 2 °C), referred to as the **equilibrium temperature**, T_{eq}. This was probably a result of evaporative cooling. Therefore, the question of whether ambient or equilibrium temperature should be used in the Newtonian equation arose. Equilibrium temperature obviously does not represent ambient temperature, but using ambient temperature as the baseline led to some problems in calculating cooling and warming constants. As Bakken (1976) showed, using ambient temperature as the baseline when it is different from equilibrium temperature does not give a straight line when $\ln(T_b-T_a)$ is plotted against time. In this situation, it becomes very inaccurate to estimate the value of the constant k because the slope of the plot is not constant (a curve is obtained). Bakken states that an error of 0.1 °C in the choice of the equilibrium temperature results in an error of 2.2 to 4.1% in the estimation of the cooling constant. He recommends using the equilibrium temperature as the baseline instead.

In the present situation, using equilibrium temperature as the baseline is justified if evaporative cooling is a purely passive process. However, care should be taken when dealing with live flies as they might use evaporative cooling as a thermoregulatory means, in order to avoid overheating for example, and in this case the rate of temperature change is not passive. The comparison between live and dead flies should make it possible to determine if the rates determined in live flies are passive or active rates of temperature change.

The present work looks not only at the problem of taking either temperature as the baseline for calculating the warming and cooling constants, but also at the effect of evaporative cooling on the warming and

cooling rates of these eristalines. To determine the effect on the cooling and warming constants of choosing one rather than the other temperature as the baseline, the constants were calculated using both baselines and were then compared. For clarity, only the results involving both calculations for dead E. *tenax* are presented here, but those for live *E. tenax* and live and dead *E. pertinax* were very similar. The cooling and warming constants for live *E. tenax* and for *E. pertinax* discussed here were calculated with the equilibrium temperature as the baseline.

The effect of evaporative cooling on the rate of warming up and cooling down will be examined, as will the effect of the size of the insect.

4.2 Materials and methods

All the flies used were caught in the wild and were kept as described in Chapter 3. Some experiments were carried out at room temperature in the laboratory, others in a controlled temperature room. Humidity was not controlled, but was around 50 % in the laboratory and in the temperature controlled room at ambient temperatures above 18 °C, and ranging from 50% to about 80 % at ambient temperatures below 18 °C in the temperature controlled room.

4.2.1 Passive rates of temperature change

The flies were cooled down in a refrigerator and then weighed (electronic balance, Sartorius Handy H160, Sartorius Ltd, UK) before being restrained in a Styrofoam-padded vice on a cooled steel stage. A small hole was made mid-dorsally in the thorax using an hypodermic needle (external diameter, 0.5 mm). Care was taken to just pierce the cuticle and not to push the needle further in the thorax. A hand-made constantan-steel thermocouple (external diameter 0.2 mm) was inserted in the hole to a depth of less than a millimetre and was secured in place using a minimal amount of glue (Copydex, Henkel Ltd, UK). The stage and fly were returned to the refrigerator until the glue had dried. The thermocouple was then inserted between two pieces of Styrofoam in a clamp held in a stand so that the fly was suspended from the thermocouple. The fly was given a small sphere of Styrofoam to hold

between its legs to prevent any flight attempts before it had reached its voluntary flight temperature. The whole apparatus was then either returned to the refrigerator or placed in a cool box (depending in which laboratory the experiment was taking place). When the fly had cooled down to about 10 °C below ambient temperature, the apparatus was transferred to a glass tank (to reduce draughts), and the thermocouple and meter (PI 8013, Portec Instruments Ltd, UK) were connected to a chart recorder (L6512, Linseis GmbH, Germany; or BS-272, Gould instruments, UK). Body temperature and ambient temperature were continuously recorded.

For the passive cooling down experiment, the flies were warmed up with a 60 W bench lamp to about 10 °C above ambient temperature before being transferred to the tank.

Each experiment was repeated twice; flies' activities during the trials were recorded. Some flies were fed during the experiment by being presented with a piece of cotton wool soaked with a 50 % sucrose solution. Most flies extended their proboscis and ingested the solution. The amount ingested was not controlled. Flies that were fed before the experiment were placed in a jar with a similar piece of cotton wool. Controls were run with flies freshly killed in a killing bottle containing some ethyl acetate. Some flies were only used live; some, only dead; others participated in both live and dead trials.

Recording errors due to heat loss from the thermocouple wires have been shown to be insignificant (Stone and Willmer 1989b).

At the end of the experiment, the flies were killed in the killing bottle, weighed again and their thoracic width measured using a binocular microscope equipped with an eye-piece graticule.

4.2.2 Statistical methods

Time and the difference between body and baseline temperature were measured directly from the curves and were plotted on an Apple Macintosh, using "Cricket Graph" version 3.

All data sets were tested for normality and, if necessary, transformed before using any parametric test. All means are given with their standard errors.

All the tests were carried out using "Minitab" version 8.2 on an Apple Macintosh.

To test for the difference in means of two populations or of matched samples, t-tests were used; n (sample size), T and p values are given in the text.

Regression was used to analyse the effect of one or more continuous variables on another one. The equation of the best fitted line is given either in the text or in the figure legend; n, p and R^2 values are given in the text.

The general linear model was employed (because of unequal sample sizes) to investigate the effect of several variables (non-continuous and continuous, in which case a covariance analysis was done) on another one. Various models were fitted, with a number of interactions between the variables. To reduce the degrees of freedom used, those interactions and predictors that were not significant and not essential for the analysis were omitted. The results are presented in tables. Each individual fly contributes one data point which is a mean of a number of values.

As explained in Chapter 3, to represent graphically the effect of a continuous variable on a second continuous variable when other factors are also involved, the residuals of the covariance analysis for the other factors on the second variable were obtained so as to control for the effect of these factors. These residuals were then plotted against the first variable.

4.3 Results

4.3.1 *E. tenax*

A/ Cooling and warming constants in dead flies

a/ Difference between ambient and equilibrium temperature

Figure 4.1 shows typical traces obtained in these experiments of the change in thoracic temperature of a live and a dead *E. tenax* warming up and cooling down.

The difference between ambient and equilibrium temperature (T_{diff}) shows a positive relationship with ambient temperature, but no relationship with mass (multiple regression for warm-up data: T_{diff} =

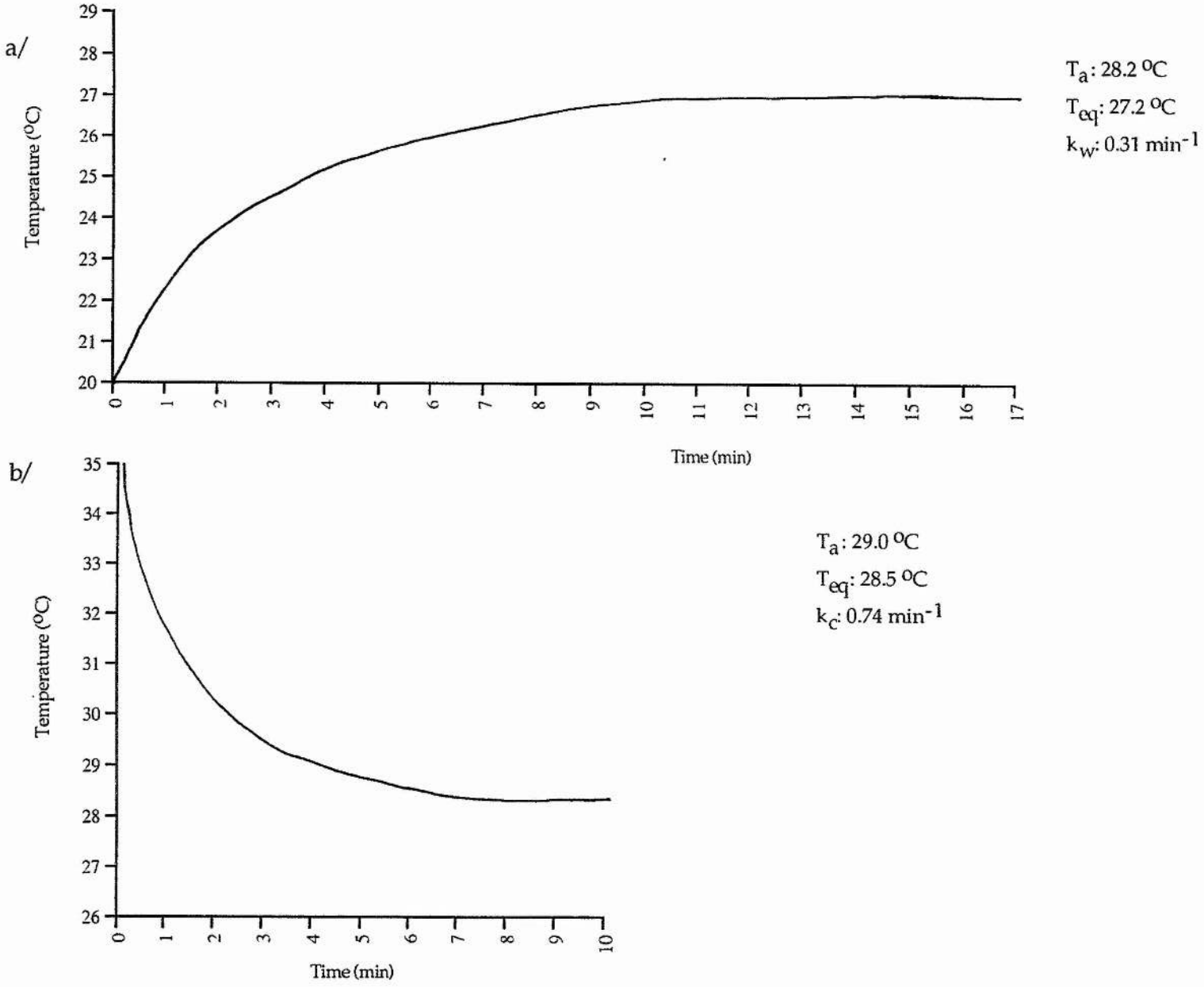


Fig. 4.1 Passive warm-up (a) and cooling down (b) traces of a live female *E. tenax*

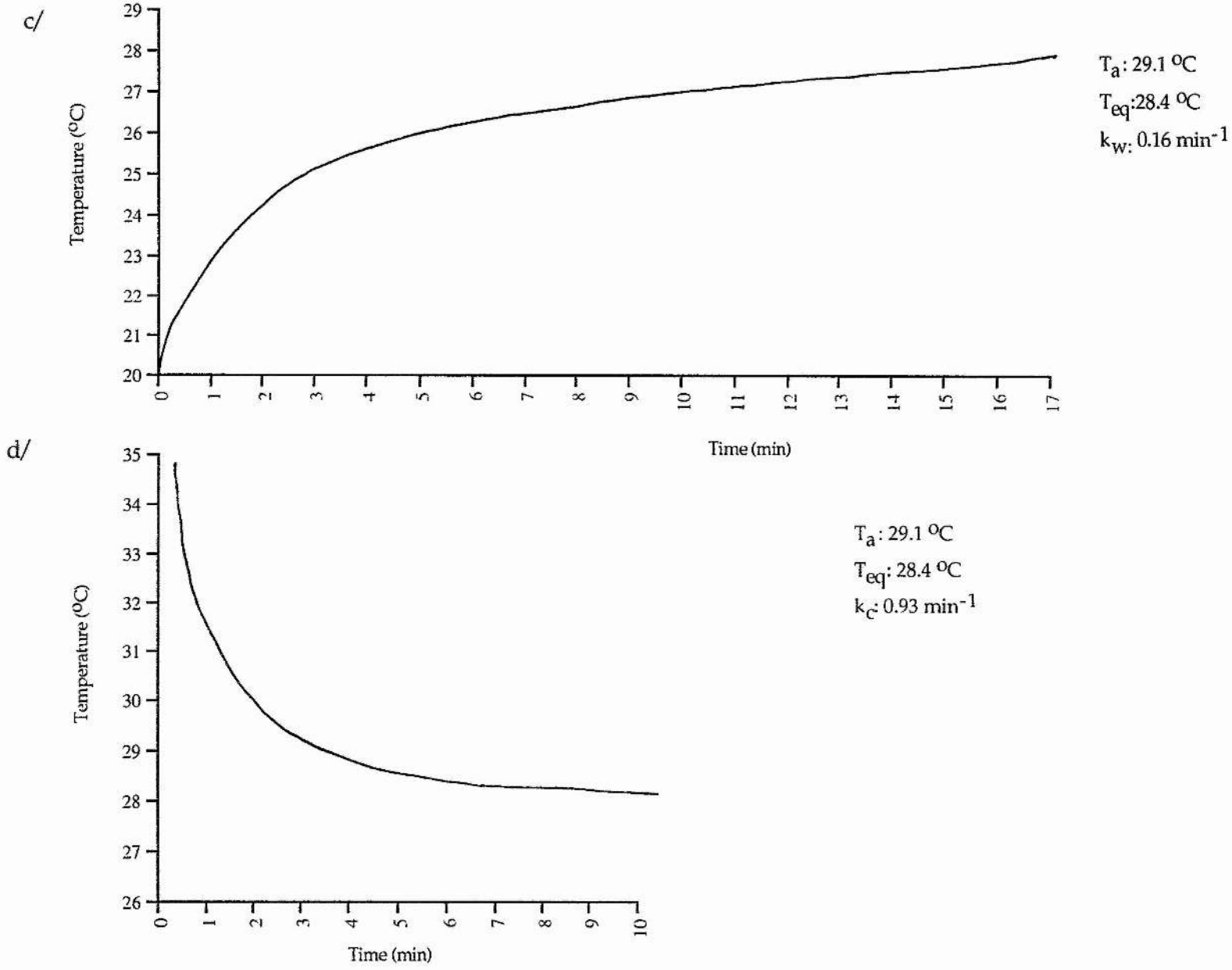


Fig. 4.1 Passive warm-up (c) and cooling down (d) traces of a dead female *E. tenax*

-0.297- 0.0022 mass + 0.056 T_a, R^2=0.55, n=34, T_a p<0.001, mass p=0.136; multiple regression for cooling down data: T_{diff} = -0.295 - 0.0016 mass + 0.050 T_a, R^2=0.59, n=36, T_a p<0.001, mass p=0.186). Of course, ambient and equilibrium temperature increase together, but the difference between the temperature at which the flies equilibrate and ambient temperature increases with ambient temperature. As ambient temperature increases, the equilibrium temperature is more and more below ambient temperature, presumably because the evaporative cooling effect increases.

b/ Equilibrium temperature as the baseline

The mean values for cooling and warming constants (using equilibrium temperature as the baseline) are:

$k_c = 0.545 \pm 0.025$ min^{-1}

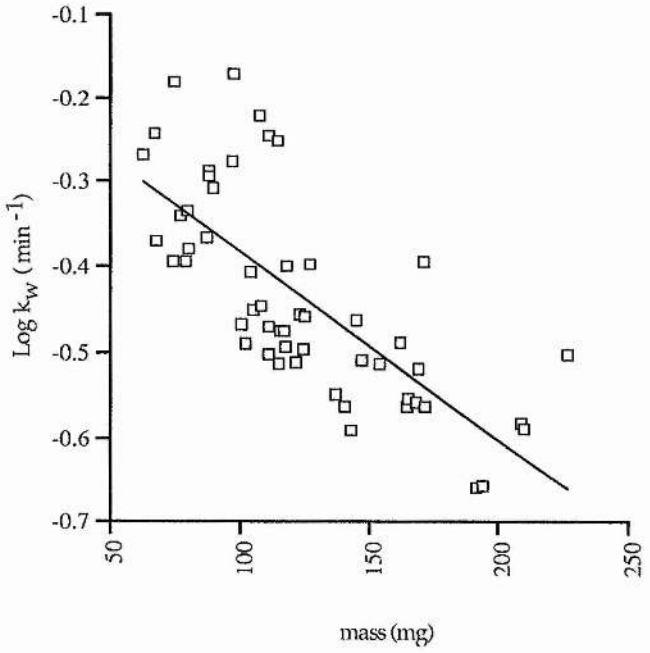
$k_w = 0.394 \pm 0.021$ min^{-1}

A covariance analysis of the effect of mass, equilibrium temperature, season and sex on the logarithm of the warming constant reveals that, once the other factors have been controlled for, there is a negative relationship between mass and the logarithm of the warming constant (Table 4.1). The results of the regression of the logarithm of the warming constant on mass and equilibrium temperature are shown in Figure 4.2a & b (n=53, R^2=0.55, mass p<0.001, T_{eq} p=0.171). Thus, as the mass increases, the warming constant decreases: heavy flies warm up more slowly than light ones. The warming constant (when calculated with equilibrium temperature as the baseline) is not under the influence of sex or season: males and females from the winter and the summer generations warm up at a similar rate.

Table 4.1 Covariance analysis on logk_w for mass, T_{eq}, season and sex in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.403	0.351	0.351	50.48	<0.001
T_{eq}	1	0.013	0.015	0.015	2.22	0.143
Season	1	0.005	0.006	0.006	0.89	0.351
Sex	1	0.001	0.001	0.001	0.18	0.677
Error	48	0.333	0.333	0.007		
Total	52	0.756				

a/



b/

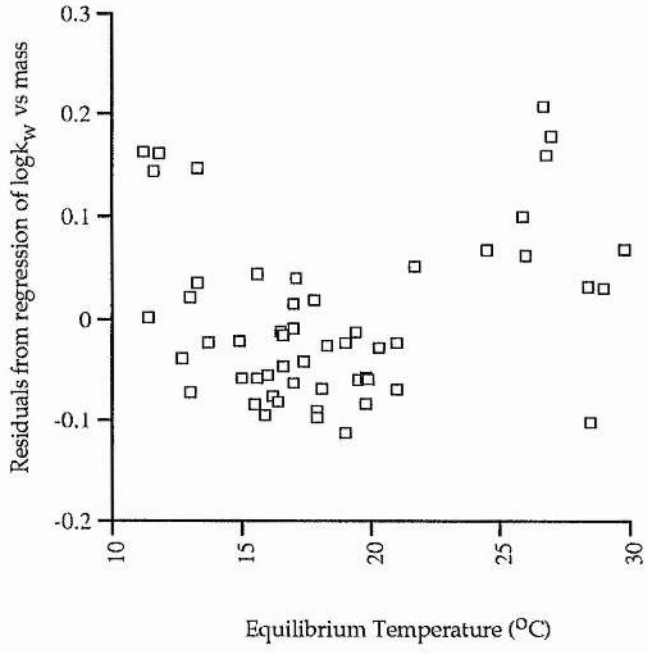


Fig. 4.2 Passive warm-up in dead *E. tenax* (T_{eq} as the baseline)

a/ Log (warming-up constant) versus mass, y = -0.002x - 0.163, r^2 = 0.533
b/ Residuals of regression of logk_w versus mass against T_{eq}

The same analysis on the logarithm of the cooling constant was carried out. Once the other factors have been controlled for, there is a negative relationship between mass and the logarithm of the cooling constant and a positive relationship between equilibrium temperature and the logarithm of the cooling constant (Table 4.2). The relationships of the logarithm of the cooling constant with mass and with equilibrium temperature are shown in Figure 4.3a & b ($n=50$, $R^2=0.57$, mass $p<0.001$, T_{eq} $p<0.001$). Again, the cooling constant (when the baseline is the equilibrium temperature) decreases with mass: heavy flies cool down more slowly than light flies. In addition, the cooling constant increases with temperature: at high temperature, flies cool down faster than at low temperature. Again sex and season have no effect on the cooling constant.

Table 4.2 Covariance analysis on $\log k_c$ for mass, T_{eq}, season and sex in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.183	0.183	0.183	22.96	<0.001
T_{eq}	1	0.229	0.165	0.165	20.75	<0.001
Season	1	0.048	0.017	0.017	2.08	0.156
Sex	1	0.055	0.001	0.001	0.01	0.922
Error	45	0.359	0.359	0.008		
Total	49	0.874				

c/ Ambient temperature as the baseline

(Cooling and warming constants were calculated for a few flies only).

The cooling and warming constants mean values (estimated with ambient temperature as the baseline) are:

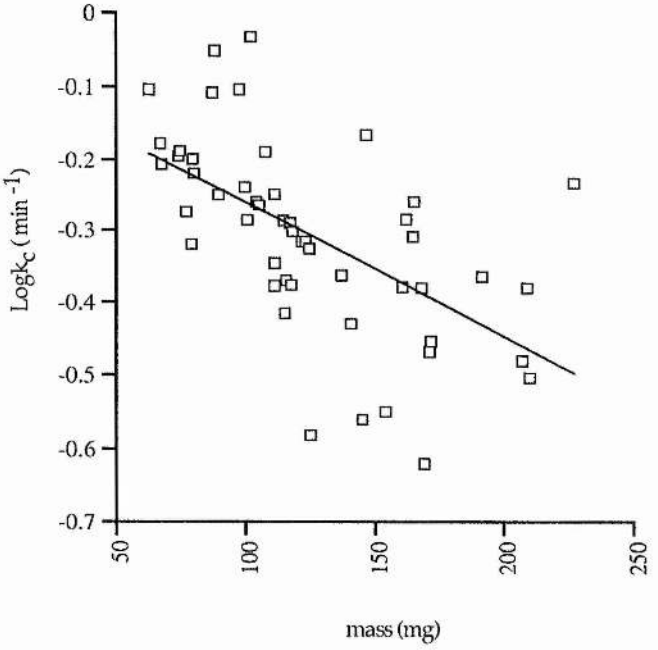
$$k_c = 0.592 \pm 0.048 \text{ min}^{-1}$$

$$k_w = 0.275 \pm 0.010 \text{ min}^{-1}$$

The same covariance analyses as above were carried out on the logarithms of the cooling and warming constants.

Once the sex and season factors have been controlled for, there is a negative relationship between mass and the logarithm of the warming constant, and there is a negative relationship between ambient

a/



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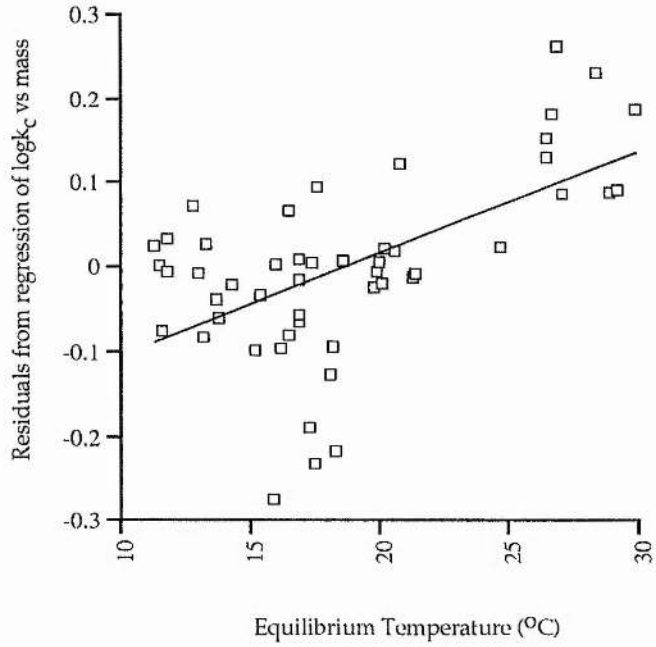


Fig. 4.3 Passive cooling in dead *E. tenax* (T_{eq} as the baseline)

a/ Log(cooling constant) versus mass, y = -0.002x - 0.074, $r^2 = 0.338$
b/ Residuals of regression of $logk_c$ versus mass against T_{eq},
y = 0.012x - 0.224, $r^2 = 0.334$

temperature and the logarithm of the warming constant (Table 4.3) The results of the regression of the logarithm of the warming constant on mass and ambient temperature are shown in Figure 4.4a & b ($n=41$, $R^2=0.50$, mass $p<0.001$, T_a $p=0.003$). Again, the warming constant decreases with mass, and there is no effect of season or sex. However, the warming constant also decreases with ambient temperature: dead *E. tenax* warm up faster at low ambient temperature than they do at high temperature when the baseline for calculating the warming constant is ambient temperature.

Table 4.3 Covariance analysis on $\log k_W$ for mass, T_a, season and sex in *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.136	0.140	0.351	27.99	<0.001
T_a	1	0.050	0.050	0.050	10.00	0.003
Season	1	0.003	0.003	0.003	0.57	0.454
Sex	1	0.005	0.007	0.007	1.36	0.251
Error	36	0.180	0.180	0.005		
Total	40	0.374				

As for the cooling constant, again there is no effect of season or sex, and once the other factors have been controlled for, there is a negative relationship between mass and the logarithm of the cooling constant; again the cooling constant decreases with mass. There is also a positive relationship between the logarithm of the cooling constant and ambient temperature. When the cooling constant is calculated with ambient temperature as the baseline, it increases with ambient temperature: flies cool down faster at high ambient temperature. The relationships of the logarithm of the cooling constant with mass and ambient temperature are shown in Figure 4.5a & b ($n=38$, $R^2=0.58$, mass $p<0.001$, T_a $p=0.001$).

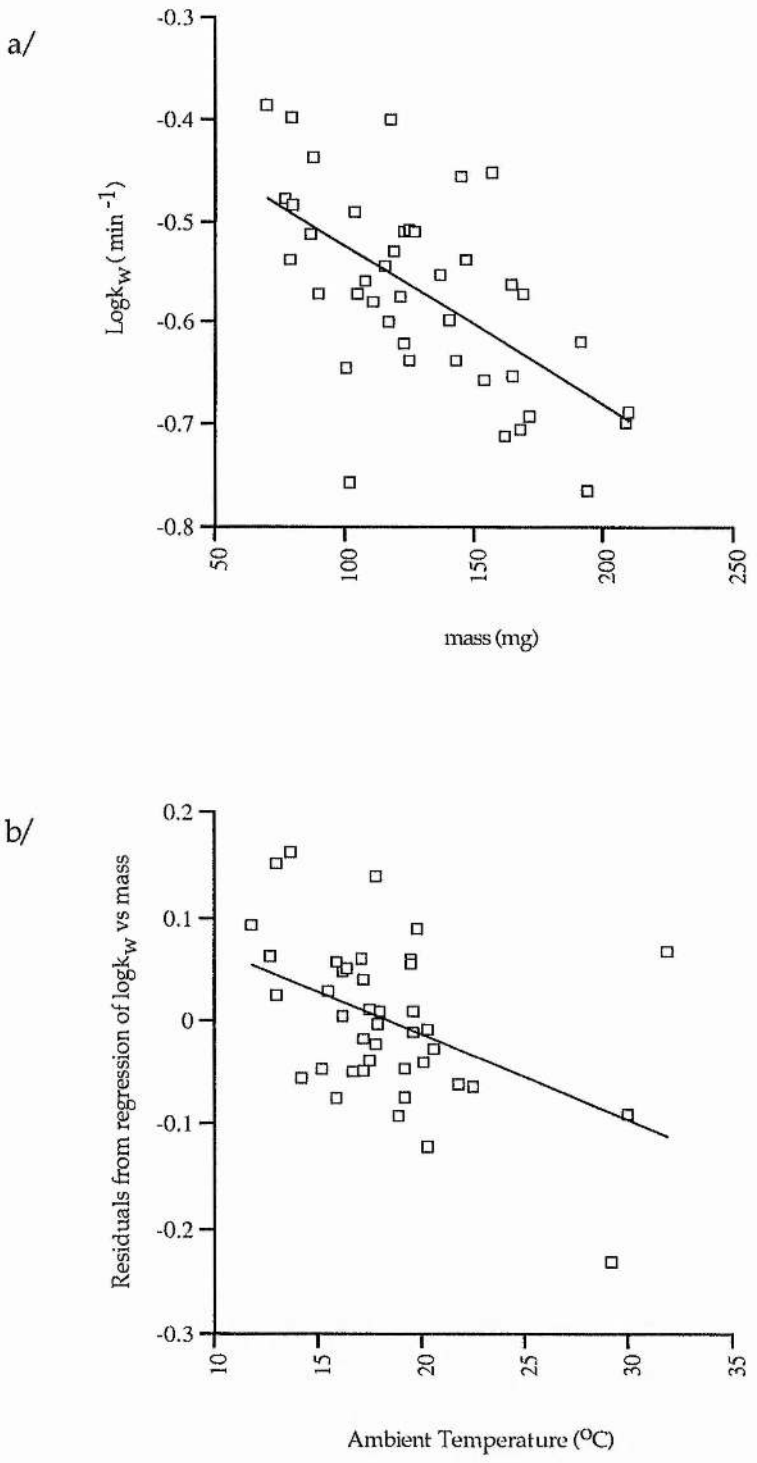


Fig. 4.4 Passive warm-up in dead *E.tenax* (T_a as the baseline)
a/ Logk$_W$ versus mass, y = -0.002x - 0.366, r^2 = 0.365
b/ Residuals of regression of logk$_W$ versus mass against T_a,
y = -0.008x + 0.152, r^2 = 0.207

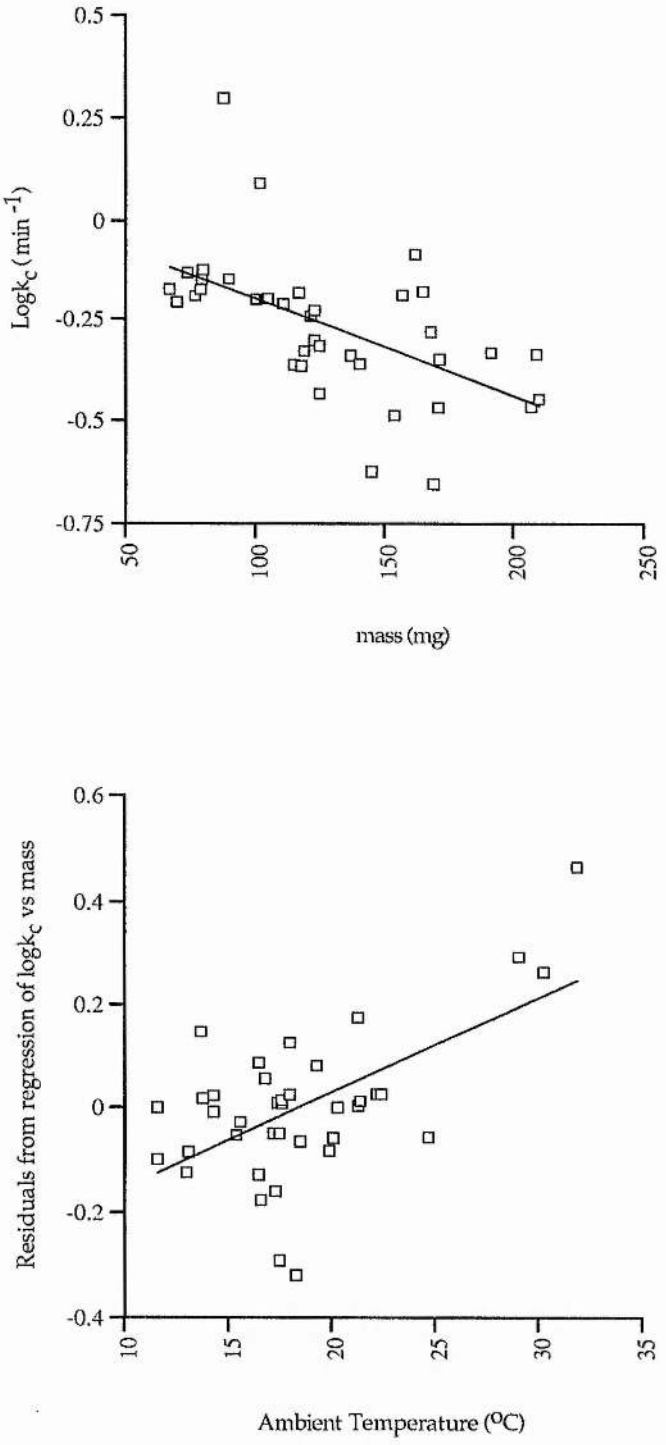


Fig. 4.5 Passive cooling down in dead *E. tenax* (T_a as the baseline)

a/ $Logk_c$ versus mass, $y = -0.002x + 0.04$, $r^2 = 0.316$
b/ Residuals of regression of $logk_c$ versus mass against T_a,
$y = 0.018x - 0.340$, $r^2 = 0.350$

Table 4.4 Covariance analysis on $logk_c$ for mass, T_a, season and sex in *E. tenax*

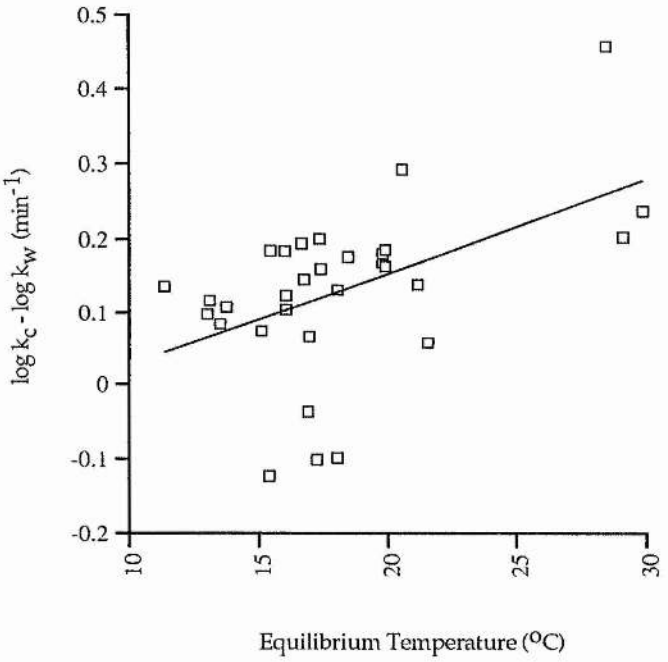
Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.362	0.201	0.201	16.08	<0.001
T_a	1	0.295	0.224	0.224	17.93	<0.001
Season	1	0.006	0.006	0.006	0.51	0.479
Sex	1	0.075	0.045	0.045	3.63	0.066
Error	32	0.400	0.400	0.013		
Total	36	1.140				

d/ Comparison of cooling and warming constants - equilibrium temperature as the baseline

The cooling and warming constants were compared with paired t-tests. The data had to be logarithmically transformed to make them normally distributed. To do a paired t-test the values of one set of data have to be subtracted from those of the other set (i.e. warming constant values had to be subtracted from cooling constant values). It was necessary to take the logarithms of the cooling and warming constants before subtracting them rather than taking the logarithm of their difference, because the logarithm of negative numbers does not exist.

A sub-sample was selected to have matched pairs (from the same flies) of cooling and warming constants (both for ambient and equilibrium temperature as baselines). When the equilibrium temperature is used as the baseline, a paired t-test on the cooling and warming constants suggests that the two constants are different; the cooling constant is larger than the warming constant ($n=31$, $T=6.31$, $p<0.0001$). A multiple regression of ($logk_c$ - $logk_w$) on equilibrium temperature and mass shows a positive relationship between ($logk_c$ - $logk_w$) and equilibrium temperature but no influence of mass ($R^2=0.24$, $n=31$, T_{eq} $p=0.007$, mass $p=0.644$; see Figure 4.6a & b). Therefore, dead *E. tenax* cool down faster than they warm up and the difference in rates increases with temperature. However, small flies are not more affected than large ones.

a/



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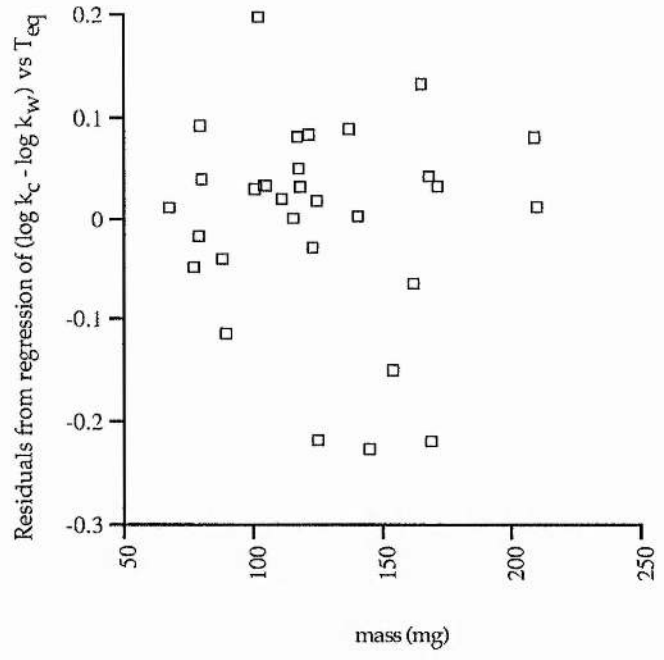


Fig 4.6 Effect of temperature and mass on the difference between the cooling and warming constants (with T_{eq} as a baseline) of *E. tenax*

a/ ($Logk_c$ - $logk_w$) against T_{eq}, $y = 0.013x - 0.099$, $r^2 = 0.238$

b/ Residuals of the regression of ($logk_c$ - $logk_w$) on T_{eq} versus mass

e/ Comparison of cooling and warming constants - ambient temperature as the baseline

A paired t-test carried out on the logarithms of the cooling and warming constants reveals that, when ambient temperature is used as the baseline, the cooling constant is again larger than the warming constant ($n=32$, $T=8.59$, $p<0.0001$). A multiple regression of ($\log k_c - \log k_w$) on ambient temperature and mass shows a positive relationship between ($\log k_c - \log k_w$) and ambient temperature, but no influence of mass ($R^2=0.51$, $n=32$, T_a $p<0.001$, mass $p=0.457$; no figure as the relationships are similar to those for T_{eq}). Dead *E. tenax* cool down faster than they warm up, and the difference is greater at high ambient temperature than at low ambient temperature. This difference is again not influenced by the size of the flies.

f/ Comparison of warming constants with equilibrium and ambient temperature as baselines

The warming constants calculated with equilibrium and ambient temperature as baselines were compared. The paired t-test reveals that there is a difference between the warming constants depending on which baseline is used for calculation ($n=31$, $T=-4.98$, $p<0.0001$): the warming constant calculated with equilibrium temperature as the baseline is larger than if calculated with ambient temperature as the baseline.

g/ Comparison of cooling constants with equilibrium and ambient temperature as baselines

Similarly, the cooling constants calculated with either equilibrium or ambient temperature as the baseline are different ($n=30$, $T=-4.55$, $p=0.0001$). The cooling constant calculated with the equilibrium temperature as the baseline is smaller than when calculated with the ambient temperature as the baseline.

B/ Cooling and warming constant in live flies

Only results for equilibrium temperature as the baseline are given here. No figures are shown here, as the relationships are very similar to those described above.

a/ Difference between ambient and equilibrium temperature

As for dead flies, the difference between ambient temperature and equilibrium temperature has a positive relationship with ambient temperature and none with mass (multiple regression for warm-up data: $T_{diff} = -0.449 - 0.00189$ mass $+ 0.0560\ T_a$, $R^2=0.50$, $n=34$, T_a $p<0.001$, mass $p=0.274$; multiple regression for cooling down data: $T_{diff} = -0.130 - 0.00279$ mass $+ 0.0393\ T_a$, $R^2=0.41$, $n=36$, T_a $p<0.001$, mass $p=0.087$). The difference between equilibrium and ambient temperature increases with ambient temperature, but the size of the flies has no effect on it.

b/ Warming and cooling constants

The mean values for cooling and warming constants in live *E. tenax* are:

$k_w = 0.399 \pm 0.019\ min^{-1}$

k_c (endothermy) $= 0.417 \pm 0.021\ min^{-1}$

k_c (lamp) $= 0.522 \pm 0.023\ min^{-1}$

Two values are shown for the cooling constant: one is the mean cooling constant after flies had endothermically warmed up, the other is the mean cooling constant after the flies had been warmed by a lamp.

A covariance analysis of the effect of mass, equilibrium temperature, season, feeding state (unfed or fed) and sex on the logarithm of the warming constant reveals that once the other factors have been controlled for, there is a negative relationship between mass and the logarithm of the warming constant (Table 4.5). The warming constant decreases with mass: heavy flies warm up more slowly than light ones. Equilibrium temperature, season, feeding state and sex have no effect on the warming constant of live *E. tenax*.

Table 4.5 Covariance analysis on $\log k_W$ for mass, T_{eq}, season, sex and feeding state in live E. *tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.238	0.237	0.237	24.37	<0.001
T_{eq}	1	0.020	0.016	0.016	1.64	0.207
Season	1	0.013	0.017	0.017	1.79	0.187
Feeding	1	0.012	0.012	0.012	1.20	0.278
Sex	1	0.002	0.004	0.004	0.43	0.514
Error	47	0.457	0.457	0.010		
Total	52	0.743				

A similar analysis on the cooling constant (artificial warm-up) suggests that once the other factors have been controlled for, there is a negative relationship between mass and the logarithm of the cooling constant and a positive relationship between equilibrium temperature and the logarithm of the cooling constant (Table 4.6). Again, season, sex and feeding state have no influence on the cooling constant. Small flies cool down faster than large ones, and live *E. tenax* which have been warmed up by a lamp cool down faster at high temperature than at low temperature.

Table 4.6 Covariance analysis on $\log k_c$ (lamp) for mass, T_{eq}, season, sex and feeding state in live E. *tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.186	0.134	0.134	18.07	<0.001
T_{eq}	1	0.073	0.064	0.064	8.66	0.006
Season	1	0.078	0.022	0.022	2.95	0.095
Sex	1	0.006	0.0003	0.0003	0.04	0.842
Feeding	1	0.007	0.007	0.007	0.92	0.344
Error	32	0.237	0.237	0.007		
Total	37	0.587				

The same analysis on the cooling constant after endothermic warm-up similarly shows a negative relationship between mass and the logarithm of the cooling constant, but no relationship between the

logarithm of the cooling constant and equilibrium temperature (Table 4.7). Again season, sex and feeding have no effect on the cooling constant, and heavy flies cool down more slowly than light ones. However, in contrast with artificially warmed flies, the cooling constant of live *E. tenax* that have warmed up endothermically is not affected by temperature: these flies do not cool down faster at high temperature.

Table 4.7 Covariance analysis on $\log k_c$ (endothermy) for mass, T_{eq}, season, sex and feeding state in live E. *tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.073	0.051	0.051	6.42	0.017
T_{eq}	1	0.025	0.025	0.025	3.07	0.090
Season	1	0.024	0.028	0.028	3.45	0.073
Sex	1	0.00005	0.00002	0.00002	0.00	0.962
Feeding	1	0.0003	0.0003	0.0003	0.704	0.841
Error	31	0.250	0.250	0.008		
Total	36	0.373				

c/ Comparison of warming and cooling constants

A sub-sample of matched pairs of cooling and warming constants was obtained. A paired t-test was carried out on pairs of the logarithms of the cooling and warming constants, showing that the cooling constant is larger than the warming constant in live *E. tenax* (n=31, T=57.41, $p<0.0001$). A multiple regression of ($\log k_c - \log k_w$) on equilibrium temperature and mass showed no influence of either of these factors ($R^2=0.13$, n=31; T_{eq} p=0.345, mass p=0.079). Therefore, live *E. tenax* cool down faster than they warm up. Small and large flies are affected in the same way, and the difference between the cooling and warming rate does not change with temperature.

d/ Comparison of cooling constants after endothermy and lamp warm-up

A covariance analysis was carried out on a sub-sample of cooling constants for flies having warmed up endothermically and flies having been warmed by a lamp (Table 4.8). The way the flies have warmed up (using external or internal heat sources) does not affect their cooling rate (warming means is not a significant factor). Ignoring season, sex and feeding state factors in the analysis does not change that result.

Table 4.8 Covariance analysis on $\log k_c$ for mass, T_{eq}, season, sex, feeding state and warming means for live E. *tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Mass	1	0.223	0.161	0.161	18.91	<0.001
T_{eq}	1	0.121	0.084	0.084	9.25	0.003
Season	1	0.019	0.018	0.018	1.77	0.152
Sex	1	0.0002	0.00001	0.00001	0.01	0.898
Feeding state	1	0.014	0.0014	0.0014	1.42	0.202
Warming means	1	0.002	0.00006	0.00006	0.01	0.935
Error	48	0.406	0.406	0.008		
Total	54	0.789				

C/ Comparison of cooling and warming constants in live and dead *E. tenax*

No difference was found between the warming constant (calculated with equilibrium temperature as the baseline) of dead and live *E. tenax* (paired t-test: n=35, T=-0.19, p=0.85). Thus, *E. tenax* warms up at the same rate irrespective of whether it is live or dead, suggesting that only passive physical processes are involved.

The cooling constant (calculated with equilibrium temperature as the baseline) of dead flies is larger than the cooling constant of live flies (paired t-test: n=36, T=-4.01, p=0.0003). Thus, dead *E. tenax* cool down faster than live flies, implying that live flies have some way of regulating their heat loss rate.

A paired t-test on the difference between ambient and equilibrium temperature in live and dead flies reveals that live *E. tenax* equilibrate at a temperature which is less depressed relative to ambient temperature than do dead flies (paired t-tests: warm-up data, n=34, T=-2.13, p=0.041; cool down data, n=36, T=-3.75, p=0.0006); the difference between ambient and equilibrium temperature is greater in dead flies.

D/ Summary

We have seen that for *E. tenax*, large flies warm up and cool down more slowly than small flies. They cool down faster at high temperature (except if they have warmed up endothermically), but their warm-up rate is not influenced by temperature. Warming and cooling constants are not affected by season, sex or feeding state.

The difference between equilibrium and ambient temperature increases with temperature in both dead and live flies, but this difference is greater in dead flies.

The warming constant calculated with equilibrium temperature as the baseline is larger than the warming constant calculated with ambient temperature as the baseline. However, the cooling constant calculated with equilibrium temperature as the baseline is smaller than the cooling constant calculated with ambient temperature as the baseline.

Both dead and live flies cool down faster than they warm up, and this difference increases with temperature.

There is no difference in the warm-up rate of dead and live flies, but dead flies cool down faster than live flies.

Explanations for all these effects are considered in the discussion.

4.3.2 *E. pertinax*

Similar problems were encountered for this species with ambient and equilibrium temperatures, and the effect of using equilibrium temperature rather than ambient temperature has similar consequences as for *E. tenax*. *E. pertinax* is also affected by water evaporation, particularly at high ambient temperature. For clarity, only the analysis using equilibrium temperature as the baseline will be presented.

Unlike the situation for *E. tenax*, mass is not a significant factor in determining the warming and cooling constants of *E. pertinax*, but thoracic width (Th_w) is significant and was used as the size factor (see Chapter 2).

A/ Warming and cooling constants in dead *E. pertinax*

The mean values for warming and cooling constants are:

$k_w = 0.608 \pm 0.05\ min^{-1}$

$k_c = 0.646 \pm 0.04\ min^{-1}$

A covariance analysis of the effect of thoracic width, equilibrium temperature, and sex on the logarithm of the warming constant was carried out. Once the other factors have been controlled for, there is a negative relationship between thoracic width and the logarithm of the warming constant (Table 4.9). The relationships of the logarithm of the warming constant with thoracic width and with equilibrium temperature are shown in Figure 4.7a & b ($n=21$, $R^2=0.45$, Th_w $p=0.001$, T_{eq} $p=0.734$). The warming constant decreases with thoracic width but is not influenced by temperature. Thus, large flies warm up more slowly than small ones.

Table 4.9 Covariance analysis on $logk_w$ for mass, T_{eq} and sex in dead *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.237	0.241	0.241	14.46	0.001
T_{eq}	1	0.002	0.001	0.001	0.08	0.778
Sex	1	0.005	0.005	0.005	0.27	0608
Error	17	0.283	0.283	0.017		
Total	20	0.527				

A similar analysis on the cooling constant reveals that once the other factor have been controlled for, there is a negative relationship between thoracic width and the logarithm of the cooling constant and a positive relationship between equilibrium temperature and the logarithm of the cooling constant (Table 4.10). The results of the regression of the logarithm of the cooling constant against thoracic width and equilibrium temperature are shown in Figure 4.8a & b ($n=21$, $R^2=0.60$, Th_w $p=0.002$, T_{eq} $p=0.002$). Again, large flies cool down more slowly than small ones,

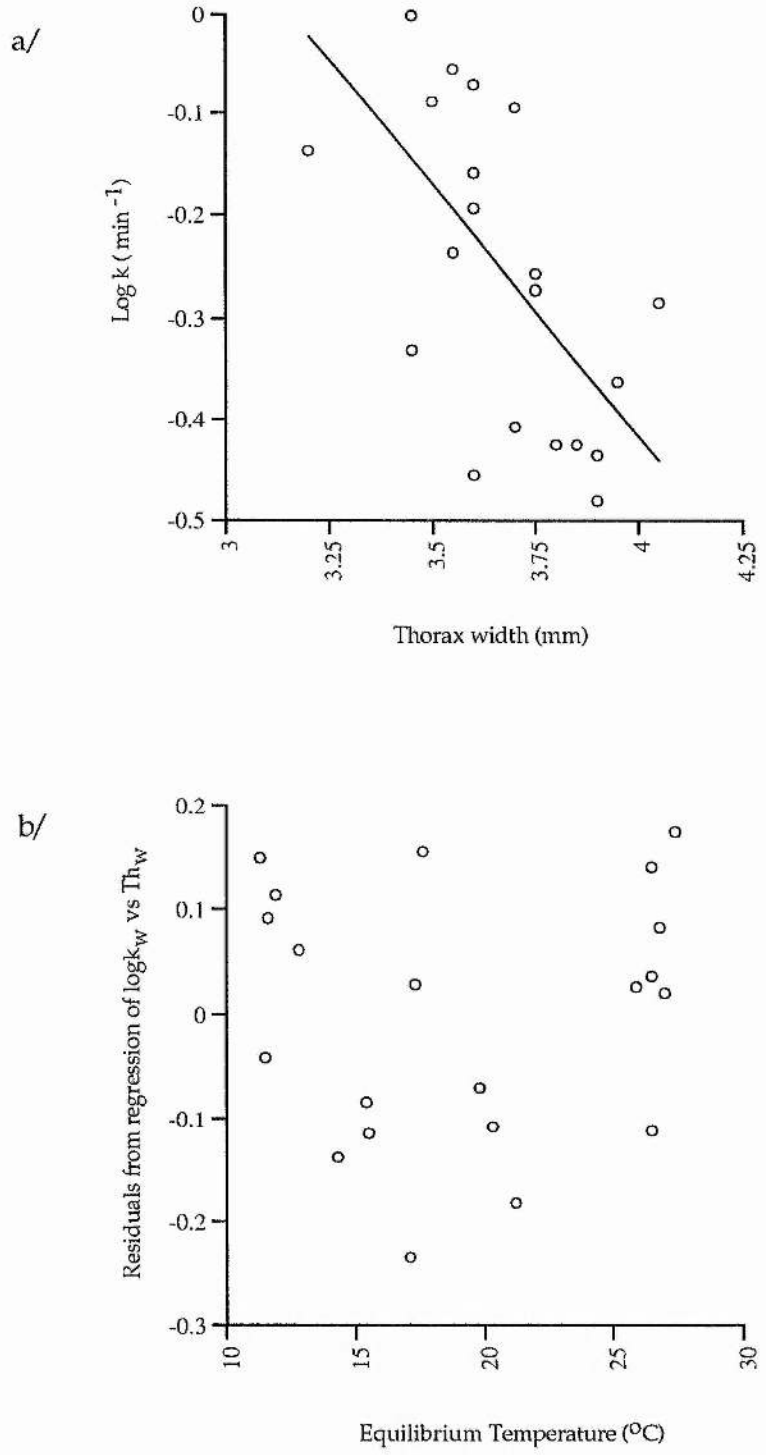
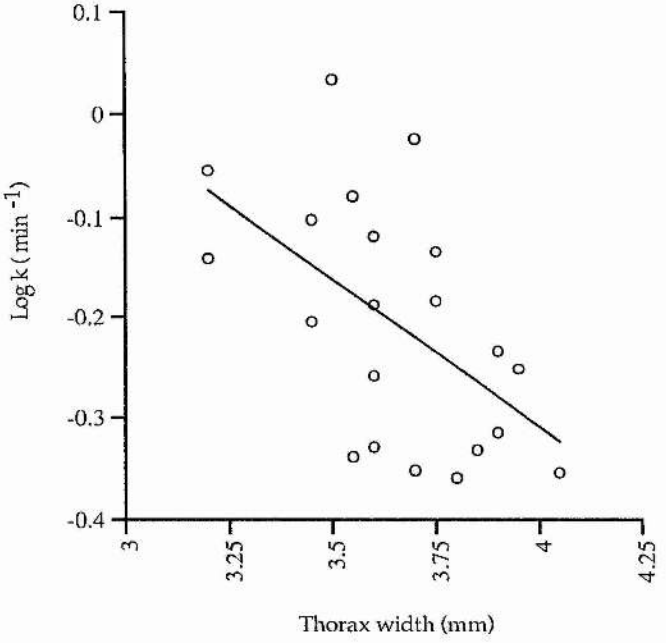


Fig. 4.7 Passive warm-up in dead *E. pertinax*

a/ Log(warming constant) versus thorax witdth, y = -0.493x + 1.557, $r^2 = 0.449$
b/ Residuals from $logk_w$ versus d against T_{eq}

a/



b/

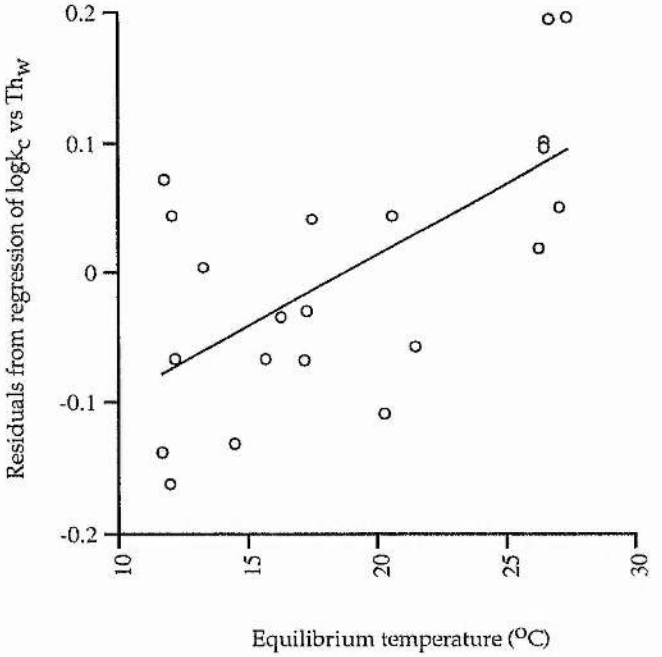


Fig. 4.8 Passive cooling in dead *E. pertinax*

a/ Log(cooling constant) versus thorax witdth, y = -0.295x + 0.871, $r^2 = 0.298$
b/ Residuals from $logk_c$ versus d against T_{eq}, y = 0.011x - 0.209, $r^2 = 0.422$

and sex has no effect on the cooling constant. Moreover, dead *E. pertinax* cool down faster at high than at low temperature.

Table 4.10 Covariance analysis on $logk_c$ for mass, T_{eq} and sex in dead *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.087	0.091	0.091	13.28	0.002
T_{eq}	1	0.084	0.084	0.084	12.25	0.003
Sex	1	0.004	0.001	0.001	0.22	0.649
Error	17	0.117	0.117	0.007		
Total	20	0.292				

A paired t-test was carried out on the logarithms of the cooling and warming constants (n=20, T=1.93, p=0.068). The difference between cooling and warming constants is not quite significant, but the cooling constant tends to be larger than the warming constant. A multiple regression of ($logk_c$ - $logk_w$) on equilibrium temperature and thoracic width shows that ($logk_c$ - $logk_w$) has a positive relationship with equilibrium temperature and a not quite significant positive relationship with thoracic width (R^2=0.39, n=20, T_{eq} p=0.018, Th_w p=0.056). Thus, the cooling constant is not significantly larger than the warming constant, but the difference between the two increases with temperature: at high temperature dead *E. pertinax* tend to cool down faster than they warm up. Also, although this is not quite significant, large flies tend to have a bigger difference between their cooling and warming constants than small flies.

B/ Warming and cooling constants in live *E. pertinax*

Relationships between the various factors are very similar to those for dead flies and are not shown in figures.

The mean values for cooling and warming constants are:

k_w=0.528 ± 0.045 min^{-1}

k_c (lamp)=0.577 ± 0.036 min^{-1}

A covariance analysis of the effect of thoracic width, equilibrium temperature, feeding state (unfed or fed) and sex on the logarithm of the

warming constant reveals that once the other factors have been controlled for, there is a negative relationship between thoracic width and the logarithm of the warming constant (Table 4.11). Again, large flies warm up more slowly than small flies, and there is no effect of temperature, season or sex on the warming constant of live *E. pertinax*.

Table 4.11 Covariance analysis on $\log k_w$ for mass, T_{eq} and sex in live *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.211	0.188	0.188	10.08	0.006
T_{eq}	1	0.003	0.0006	0.0006	0.03	0.858
Feeding	1	0.039	0.039	0.039	2.08	0.167
Sex	1	0.003	0.0006	0.0006	0.03	0.862
Error	17	0.317	0.317	0.018		
Total	21	0.571				

The same analysis on the cooling constant (after warming up by a lamp) shows that once the other factors have been controlled for, there a positive relationship between equilibrium temperature and the logarithm of the cooling constant. The negative relationship between thoracic width and the logarithm of the cooling constant is not quite significant (Table 4.12), thus large flies do not cool down significantly more slowly than small ones, but live *E. pertinax* cool down faster at high temperature.

Table 4.12 Covariance analysis on $\log k_c$ for mass, T_{eq} and sex in live *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.039	0.040	0.040	3.83	0.068
T_{eq}	1	0.065	0.065	0.065	6.25	0.024
Sex	1	0.032	0.027	0.027	2.56	0.129
Feeding	1	0.00007	0.0012	0.0012	0.11	0.743
Error	16	0.167	0.167	0.010		
Total	20	0.304				

Not enough data on the cooling constant after endothermy could be obtained to analyse them.

A paired t-test was carried out on the logarithms of the cooling and warming constants (n=19, T=0.78, p=0.44). There is no difference between the cooling and the warming constants in live *E. pertinax*. A multiple regression of ($logk_c$ - $logk_w$) on equilibrium temperature and thoracic width shows a positive relationship between ($logk_c$ - $logk_w$) and these two factors (R^2=0.49, n=19; T_{eq} p=0.005, Th_w p=0.041). Therefore, live *E. pertinax* warm up and cool down at the same rate, but the difference between warming and cooling constants increases with temperature and with thoracic width.

C/ Comparison of warming and cooling constants in live and dead *E. pertinax*

The warming constant of dead *E. pertinax* is larger than that of live ones (paired t-test: n=18, T=2.91, p=0.0097): dead E. *pertinax* warm up faster than live ones.

The difference between the warming constants is positively correlated with the percentage of mass lost by the flies during the experiments (R^2=0.37, n=14, percentage of mass loss p=0.020). The more the mass was reduced during the experiment, the bigger the difference between the warming constants of dead and live flies.

A paired t-test shows that the cooling constant of dead flies is larger than the cooling constant of live flies (n=20, T=2.41, p=0.026): dead flies cool down faster than live flies.

An Anova analysis on the difference between equilibrium and ambient temperature for all four conditions (warming up and cooling down in live and dead flies) reveals that there is a significant difference between them (n=76, $F_{(3,72)}$=4.83, p=0.004). This result is due to the difference between equilibrium and ambient temperature of live flies cooling down being smaller than the difference between equilibrium and ambient temperature of the other three conditions (which are all similar).

The difference between equilibrium and ambient temperature for dead *E. pertinax* warming up is not different from that of live flies at high ambient temperature (> 17 °C) (paired t-test, n=9, T=0.91, p=0.39), but the

difference between equilibrium and ambient temperature of dead flies is larger than that of live flies at low ambient temperature (<17 °C) (paired t-test, n=10, T=2.79, p=0.0021). Thus, at low temperature dead *E. pertinax* used in warming up experiments equilibrate at a temperature which is lower relative to ambient temperature than do live flies. At high temperature there is no difference between live and dead flies.

The difference between equilibrium and ambient temperature for cooling dead flies is larger than for cooling live flies at both high and low ambient temperatures (paired t-tests; high ambient temperature: n=14, T=3.82, p=0.0021; low T_a: n=13, T=3.58, p=0.0038). Therefore dead *E. pertinax* used in cooling down experiments equilibrate at a temperature which is lower relative to ambient temperature than do live flies.

D/ Summary

Large E. *pertinax* warm up and cool down more slowly than small flies. The warming and cooling constants are not affected by sex or feeding state. Warm-up is not affected by ambient temperature, but cooling down does increase with temperature. *E. pertinax* does not cool down faster than it warms up (although there is a tendency in this direction in dead flies). However, dead flies warm up and cool down faster than live ones.

Live flies used for the cooling down experiment equilibrate at a temperature higher relative to ambient temperature than dead flies, or than dead and live flies used for the warm up experiment. Also, dead flies used for the warm-up experiment equilibrate at a temperature lower relative to ambient temperature than live flies, but only at low ambient temperature. These points are considered further in the discussion.

4.3.3 Comparison of the two species

No interaction between species and mass was detected in the following analyses, so this factor was omitted.

A/ Warming up in dead flies

A covariance analysis (Table 4.13) investigated the effect the logarithm of the warming constant of thoracic width, mass, equilibrium temperature, species and the interactions between species and thoracic width and between species and equilibrium temperature. The logarithm

of the warming constant has a negative relationship with mass and thoracic width. The relationship between the logarithm of the warming constant and the thoracic width is different in the two species, but the species do not differ in their relationship between the logarithm of the warming constant and equilibrium temperature. In addition, the warming constant is influenced by the species factor: from the fitted means, it is clear that when mass and thoracic width have been controlled for, *E. tenax* warms up more slowly than *E. pertinax*.

Table 4.13 Analysis of covariance on $\log k_w$ for Th_w, mass, T_{eq} and species in dead *E. tenax* and *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.546	0.173	0.173	16.88	<0.001
Mass	1	0.253	0.204	0.204	19.86	<0.001
T_{eq}	1	0.054	0.006	0.006	0.57	0.452
Species	1	0.081	0.136	0.136	13.25	0.001
Species*Th_w	1	0.120	0.122	0.122	11.90	0.001
Species*T_{eq}	1	0.004	0.004	0.004	0.37	0.545
Error	64	0.656	0.656	0.010		
Total	70	1.714				

Fitted means for species

	Mean (min^{-1})	Stdev (min^{-1})
E. tenax	-0.405 (mean k=0.398)	0.015
E. pertinax	-0.371 (mean k=0.427)	0.030

B/ Cooling down in dead flies

A covariance analysis on the logarithm of the cooling constant for thoracic width, mass, equilibrium temperature, species, and the interactions between species and thoracic width and between species and equilibrium temperature was carried out (Table 4.14). The logarithm of the cooling constant has a negative relationship with both mass and thoracic width: large flies cool down more slowly than small flies. In addition, the cooling constant has a positive relationship with temperature: flies cool down faster at high temperature. The relationship between the logarithm of the cooling constant and thoracic width is different in the two species,

but the relationship between the logarithm of the cooling constant and temperature is the same in the two species. Moreover, there is a significant species effect on the cooling constant of these dead eristalines: *E. tenax* has a greater cooling constant than *E. pertinax*.

Table 4.14 Analysis of covariance on $\log k_c$ for Th_w, mass, T_{eq} and species in dead *E. tenax* and *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.178	0.047	0.047	6.22	0.015
Mass	1	0.108	0.112	0.112	14.70	<0.001
T_{eq}	1	0.338	0.222	0.222	29.21	<0.001
Species	1	0.006	0.058	0.058	7.67	0.007
Species*Th_w	1	0.052	0.054	0.054	7.08	0.010
Species*T_{eq}	1	0.004	0.004	0.004	0.49	0.486
Error	60	0.457	0.457	0.007		
Total	66					

Fitted means for the logarithm of the cooling constant

	Mean (min^{-1})	Stdev (min^{-1})
E. tenax	-0.281(mean k=0.525)	0.014
E. pertinax	-0.291 (mean k=0.513)	0.026

C/ Warm-up in live flies

The same covariance analysis on the warming constant of live flies (Table 4.15) shows that there is a negative relationship between the logarithm of the warming constant and mass and thoracic width: small flies warm up faster than large ones. There is no relationship between the logarithm of the warming constant and temperature. The relationship between the logarithm of the warming constant and thoracic width is different in the two species. The warming constant of live flies is affected by the species predictor: once mass and thoracic width have been controlled for, *E. pertinax* warms up faster than *E. tenax*.

Table 4.15 Analysis of covariance on $logk_w$ for Th_w, mass, T_{eq} and species in live E. *tenax* and *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.559	0.211	0.211	16.26	<0.001
Mass	1	0.043	0.056	0.056	4.33	0.042
T_{eq}	1	0.031	0.0009	0.0009	0.07	0.790
Species	1	0.027	0.073	0.073	5.60	0.021
Species*Th_w	1	0.060	0.065	0.065	5.02	0.029
Species*T_{eq}	1	0.007	0.007	0.007	0.56	0.458
Error	62	0.804	0.804	0.013		
Total	68	1.531				

Fitted means for the logarithm of the warming constant

	Mean (min^{-1})	Stdev (min^{-1})
E. tenax	-0.432(mean k=0.372)	0.018
E. pertinax	-0.418 (mean k=0.389)	0.035

D/ Cooling down in live flies

The same covariance analysis on the cooling constant of live flies (Table 4.16) was carried out. The cool down constant has a negative relationship with mass but not with thoracic width: light flies cool down faster than heavy ones, but thoracic width has no influence on the cooling constant of these live eristalines. There is a positive relationship between the logarithm of the cooling constant and equilibrium temperature: live flies cool down faster at high temperature. However, there is no difference between the species in their cooling constants or their relationships between the logarithm of the cooling constant and thoracic width and between the logarithm of the cooling constant and equilibrium temperature.

Table 4.16 Analysis of covariance on logkc for Th_w, mass, T_{eq} and species in live *E. tenax* and *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
Th_w	1	0.090	0.012	0.012	1.39	0.244
Mass	1	0.118	0.124	0.124	14.73	<0.001
T_{eq}	1	0.187	0.150	0.150	17.90	<0.001
Species	1	0.004	0.017	0.017	2.00	0.164
Species*Th_w	1	0.021	0.019	0.019	2.29	0.137
Species*T_{eq}	1	0.002	0.002	0.002	0.23	0.631
Error	48	0.404	0.404	0.008		
Total	54	0.827				

E/ Mass loss during the experiments

The mass loss presented here are for flies which were not fed during the experiment.

The mean loss of mass are:

- *E. tenax*: 14.53 ± 1.01 mg (range: 2.5 - 28.0 mg)
- *E. pertinax*: 16.84 ± 2.30 mg (range: 3.0 - 38.0 mg)

The mean percentage of mass loss (relative to initial mass) are:

- *E. tenax*: 11.67 ± 0.86% (range: 2.5 - 24.0%)
- *E. pertinax:* 16.92 ± 2.12% (range: 3.0 - 42.9%)

F/ Summary

Heavy and large flies (except live cooling flies) exchange heat passively with their environment more slowly than light and small flies.

Both species have similar relationships between their cooling and warming constants and temperature and between the constants and mass. However, they have different relationships between the cooling and warming constants and thoracic width.

In addition, live and dead *E. pertinax* warm up faster than live and dead *E. tenax;* dead *E. pertinax* cool down more slowly than dead *E. tenax;* but there is no difference between the two species regarding their cooling constant when alive.

4.4 Discussion

4.4.1 The use of ambient or equilibrium temperature

Why are ambient temperatures problematic as a baseline? Difficulties in calculating cooling constants and, in particular, warming constants were encountered because the thorax temperature at which the flies equilibrate is lower than ambient temperature. It is likely that this phenomenon stems from the cooling effect of water evaporation, because it was shown that the difference between equilibrium and ambient temperature increases with ambient temperature, as would be expected because the rate of water loss (and thus evaporative heat loss) is independent of the difference between body and ambient temperature, but depends on body temperature (May 1995). Moreover, it was shown in Chapter 3 that *E. tenax* and *E. pertinax* are quite "leaky" flies. Although their rate of water loss is in the range of that of other mesic insects, and in particular flies, they are in the upper part of the range. Thus, these eristalines lose water at a high rate. Do they lose enough water to account for the temperature depression to equilibrium temperature? It was calculated that, for a 100 mg fly, to keep its body temperature 2 °C below ambient temperature would require the loss of 0.28 mg of water per hour (assuming that the specific heat capacity of the animal body is 0.8 cal g^{-1} $°C^{-1}$ and that the heat of evaporation of water is 580 cal g^{-1}; Schmidt-Nielsen 1990, Withers 1992). This is well within the range of passive water losses described in Chapter 3.

Cooling and warming constants correspond to the gradient of the line of the plot of $\ln(T_{th}-T_{ref})$ against time - T_{ref} being either equilibrium or ambient temperature. When ambient temperature is taken as the baseline, the thoracic temperature of the warming flies does not reach this baseline (see Figure 4.9). Thus, when $\ln(T_{th}-T_a)$ is plotted against time, instead of a straight line, a curve is obtained, because the time taken to get a unit of temperature closer to ambient temperature increases as the fly warms up, but not exponentially: this duration tends towards infinity because the fly never reaches ambient temperature. The same plot would give a straight line if the fly reached the baseline temperature (assuming that the only cause of warming up is the temperature difference between the fly and its

environment) because the time taken to get a unit of temperature closer to ambient temperature as the fly warms up increases exponentially. Therefore, the estimates of warming constants using ambient temperature are not accurate and vary depending on which part of the curve is used for the estimation. The problem is different for the cooling constant because thoracic temperature overshoots the baseline (Fig. 4.9). Plots of $\ln(T_{th}-T_a)$ versus time also result in curves. However, because the body temperature used varied from 7 °C to 1°C above ambient temperature, the plot obtained is in the straighter part of the curve. The curve really bends more strongly when body temperatures below ambient temperature are included (see also Bakken 1976). Thus, less problems were encountered for the estimation of the cooling constant (but this does not imply that the cooling constants thus calculated are the "correct" ones).

Calculating the warming and cooling constants with the equilibrium temperature as reference solved these problems. Warming constants calculated with ambient temperature as the baseline are smaller than warming constants calculated with equilibrium temperature as the baseline. In contrast, estimates for the cooling constant calculated with ambient temperature as the baseline are larger than estimates calculated with equilibrium temperature as the baseline. This is to be expected, as for a warming fly it takes more time to reach the same $(T_{th}-T_{ref})$ if ambient rather than equilibrium temperature is the reference temperature: a smaller warming constant is obtained (Fig 4.9). Conversely, a cooling fly reaches the same $(T_{th}-T_{ref})$ faster if ambient rather than equilibrium temperature is the baseline: the cooling constant is larger. Thus the constants are artificially decreased or increased if ambient temperature is used as the baseline. Using equilibrium temperature as the baseline gives a more accurate estimate of these constants. This is in accordance with the findings of Bakken (1976).

In the following discussion, the effects on the constants of taking either baseline will be explained. For further analyses, if it is possible to measure the equilibrium temperature, results using both ambient and equilibrium temperature will be compared. In some situations (for example in the "grab and stab" experiment in Chapter 5), ambient temperature will be used as the reference because equilibrium temperature cannot be measured.

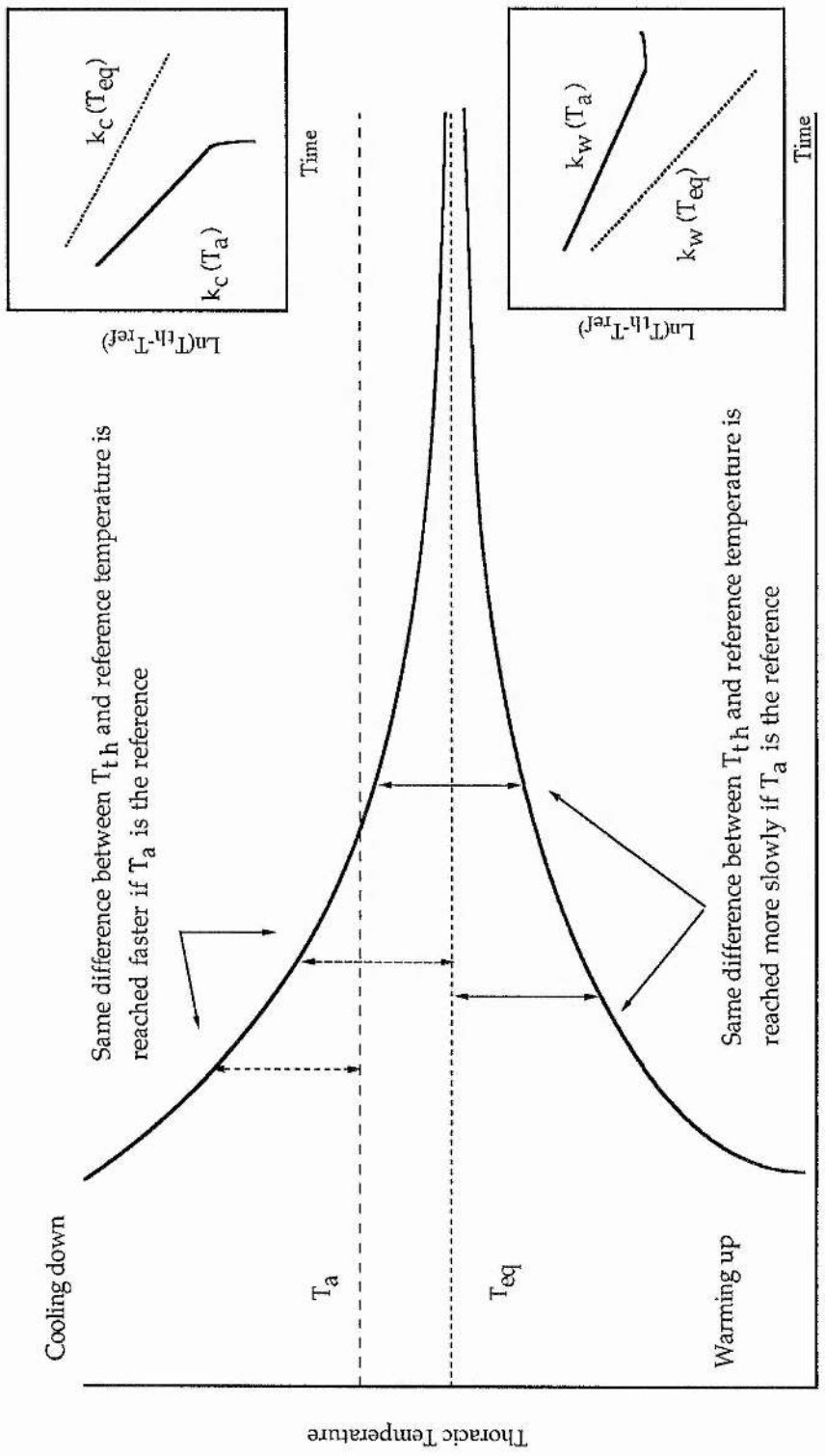


Fig. 4.9 Effect of water evaporation on calculating the constants of temperature change

4.4.2 Thermal constraints and evaporative cooling

No effect of season, feeding state or sex on cooling and warming constants could be demonstrated. Thus, there does not seem to be any effect of structural (cuticular or pile) modification between the generations of *E. tenax* which face different climatic conditions. Nor does there seem to be any difference between males and females in that respect for either species.

Using equilibrium temperature as the baseline, a positive relationship between the cooling constant and "external" temperature was demonstrated in both species, dead and live. No relationship was found between the warming constant and "external" temperature. Therefore, these eristalines cool down faster at high temperature, but their warm-up is not affected by ambient temperature. This result can be explained by the inevitable increase in water evaporation at high temperature, increasing the cooling rate. Using equilibrium temperature as the reference is sufficient to eliminate the influence of temperature on the warming but not on the cooling constant. At a particular ambient temperature, the effect of water evaporation is that the fly equilibrates at a temperature that is lower than ambient temperature. Using equilibrium temperature rather than ambient temperature as the baseline for calculating the warming and cooling constants corrects that temperature depression at that particular temperature. However, cooling flies are also subject to "extra" water evaporation because their body is well above ambient temperature as it cools down. Warming flies are only affected by water evaporation associated with the particular ambient temperature at which they are being tested, as their body temperature is lower than ambient temperature. Therefore, using equilibrium temperature as the baseline corrects the effect of ambient temperature on the warming constant, but only partly does so on the cooling constant. The negative relationship between the warming constant and temperature which is found when ambient temperature is used as the baseline (in dead *E. tenax*) disappears. The "extra" water evaporation that affects the cooling constant is more important at high than at low temperature, hence the positive relationship of the cooling constant and temperature.

The reason why the cooling constant after endothermic warm-up in *E. tenax* does not show any relationship with temperature is probably that the flies do not warm-up endothermically at high temperature: the cooling

constant could not be determined at high temperature. Thus, that part of the range which has the more influence on the cooling constant is absent from the data. The fact that humidity was not controlled and was higher at low than at high temperature probably adds to the effect of water evaporation.

4.4.3 Comparison of cooling and warming constants

There does not seem to be any reason why the cooling constant should be different from the warming constant in dead insects; Willmer and Unwin (1981) did not find any difference in the warming and cooling rates of a sample of freshly killed insects (which included *E. pertinax*). However, this is not necessarily the case for live animals as they may physiologically alter their rates of warming up and cooling down. For example, iguanas, *Amblyrhynchus cristatus*, (Morgareidge and White 1969) tend to warm up faster than they cool down by adjusting their blood flow to facilitate heating and retard cooling. These animals show a peripheral vasodilatation during heating (helps gaining heat from the environment) and vasoconstriction during cooling (helps reducing heat loss to the environment). In contrast here, in *E. tenax* the cooling constant is larger than the warming constant.

The difference between cooling and warming constants becomes larger with increasing temperature (in dead flies), supporting further the hypothesis that evaporation is responsible for these results. Water loss regulation (see below) is probably responsible for the lack of correlation of the difference between the cooling and warming constants with temperature in live *E. tenax*. In *E. pertinax*, the cooling constant is not quite significantly larger than the warming constant in dead specimens. The difference between cooling and warming constants does increase with temperature, suggesting that the same effect as for *E. tenax* exists, but this could not be demonstrated probably because of a small sample size and possibly a smaller difference between the constants (mean $\log k_c$-$\log k_w$=0.130 in dead *E. tenax*, mean $\log k_c$-$\log k_w$=0.047 in dead *E. pertinax*). The absence of a difference between the warming and cooling constants in live *E. pertinax* suggests some active regulation of water loss.

The difference between cooling and warming constants is not influenced by mass in *E. tenax*: smaller flies do not seem to be more affected by the cooling effect of water evaporation than larger flies or vice

versa. In *E. pertinax*, the difference between the constants increases with thoracic width: larger flies are more affected by this effect of water evaporation than small flies. This is in accordance with the findings of Chapter 3 where it was shown that in live flies the rate of water loss is positively correlated with size.

In *E. tenax*, there is no difference between the cooling constant after endothermy and the cooling constant after lamp warm-up. After lamp warm-up, the fly cools from the whole body. After endothermy, the fly either cools from the whole body (if heat is transferred to the abdomen with haemolymph) or only from the thorax (if the heat produced by the flight muscles remains in the thorax). If the fly cools down from the thorax only, the cooling constant should be different from the cooling constant measured after artificial warm-up (cooling from whole body) because the heat would be kept in the thorax and would not be dissipated through the abdomen. Here, it seems that *E. tenax* cools from the whole body (heat is transferred to the abdomen) because the cooling constant after endothermic warm-up is the same as the cooling constant after the fly has been warmed up by a lamp (whole body).

4.4.4 Comparison of warming and cooling constants between live and dead flies

Endothermy did not seem to affect the warming constant of live flies (k_W is not different in dead and live animals). May (1976a) found that the cooling constant of live insects is larger than the cooling constant of dead ones. He suggested that this is the result of haemolymph circulation increasing heat loss in live specimens. Here, the cooling constant of live flies is smaller than that of dead flies. Endothermy could be responsible, but another explanation seems more likely. It was shown that the difference between equilibrium and ambient temperature in live flies is smaller than that in dead flies. Thus, it seems that these live syrphids are able to restrict their water loss to some extent and thus reduce the difference between equilibrium and ambient temperature (however care should be taken with the interpretation as Hadley (1994) showed that dead insects lose water faster than live ones, even if their spiracles are blocked). This is probably achieved by controlling the opening of the spiracles. If water evaporation is limited, the acceleration of cooling down at high temperature is reduced, and the cooling constant is lowered. For

the warming constant, the effect of evaporative cooling is already controlled by using the equilibrium temperature as the baseline: no difference is expected between the warming constants of live and dead flies. It is probable that the alteration of the cooling constant is just a consequence of the control of water evaporation which is aimed at conserving water. In fact, if it were the rate of cooling down that were controlled, an increase at high temperature might be expected rather than a decrease.

For *E. pertinax*, the difference between equilibrium and ambient temperature is the smallest for live flies cooling down, whereas it is similar in the other three conditions. This suggests that water loss is being controlled in cooling live flies (as in *E. tenax*). Furthermore, it seems that water evaporation is controlled at all temperatures in cooling flies, but only at low temperature in warming flies (as the difference between equilibrium and ambient temperature of warming live flies is smaller than that of warming dead flies at low ambient temperature only). In addition, it is worth noting that warming trials in live flies were always performed first. It can be suggested that during the warming trials, either through stress or through voluntarily increasing water losses to avoid overheating, *E. pertinax* does not restrict its water losses at high temperature. It is possible that the loss of water that resulted was so important (up to 43% decrease of the body mass was recorded during the experiments - although this mass loss does not represent water losses only) that when the cooling trials were done, the flies reduced their water losses.

As for *E. tenax*, no difference between the warming constants of live and dead flies is expected. Surprisingly however, the warming constant of dead flies is larger than the warming constant of live flies. Mass loss could offer an answer for this increase in warming constant (similar mass losses in *E. pertinax* represent a higher percentage relative to body mass than in *E. tenax* and might be reflected in the warming constants of the former species). Dead flies were lighter than live flies, and the difference between the warming constants of dead and live flies is positively correlated with the percentage of mass loss. The fact that mass is not a significant factor in predicting the warming and cooling constants in *E. pertinax* does not contradict this suggestion, because here the mass effect is for paired data (that is, the reduction in mass of individual flies). It is easier to pick up the effect of a mass decrease in individual subjects that do not change in

other respects than across a number of subjects all differing for other parameters as well (e.g. thoracic width). The decrease in mass would also lead to an increase in the cooling constant of dead flies. Thus, the fact that the cooling constant of dead flies is larger than the cooling constant of live flies is probably due to a combination of both a mass decrease and water loss restriction in live flies.

When using dead flies for estimating the warming and cooling constants, it is assumed that these constants represent the passive heat exchange between the animal and its environment; no physiological process is involved, and they purely depend on physical processes. However, these constants might differ in live subjects. A live insect has its metabolism working, so heat is produced even if the animal is resting; and haemolymph is circulating around its body, potentially dissipating heat. Also, a dead insect can have its spiracles either open or closed, and they remain that way; in a live insect, they open and close regularly (even if not actively controlled for water balance). Therefore, the cooling and warming constants of live and dead animals might be different even if no active physiological control takes place. In addition, live insects can actively employ physiological processes (such as endothermy, closing their spiracles to limit water evaporation, or opening them to facilitate cooling down) to control their thermal balance and their water content. Thus, using live animals provides a more realistic view of their thermal constraints, but incurs the risk of not looking at purely physical aspects only but at physiological ones as well. Testing live animals in different conditions (e.g. temperature and humidity) can help to distinguish between these processes. Here, only temperature could be varied but it did allow me to make some tentative inferences about the control of water evaporation by these eristalines and its effect on the warming and cooling constants.

It is normally assumed that evaporative cooling is negligible in insects, except in a few cases when there is a threat of overheating (see Chapter 3). Here, even if it has not been specifically demonstrated that these flies use evaporative cooling to cool down actively when heat stressed, water evaporation (probably passive) clearly has an effect on their thermal balance. It seems that live flies actively reduce their water loss during these experiments, and this indirectly affects the warming and cooling constants.

4.4.5 Effect of size on the warming and cooling constants

There is a strong negative relationship between mass and the warming and cooling constants in *E. tenax* and between thoracic width and the warming and cooling constants in *E. pertinax* (thoracic width is also a good predictor for warming and cooling constants in *E. tenax*, but not as strong as mass). Thus, one size factor which predicts a variable well in one species does not necessarily do so in another. Both mass and linear dimensions are affecting the rate at which an object changes temperature as both surface area to volume ratio and specific heat of its contents are important. As shown in Chapter 2, for the same thoracic width, *E. pertinax* females are heavier than males, and the difference comes from the abdomen (i.e. the two sexes are the same shape regarding their thorax). In *E. pertinax*, females with the same thoracic width as males have the same surface area to volume ratio but their body mass (because of abdominal contents) differs. As the change in temperature was measured for the thorax, it is likely that thoracic width is a more important factor in influencing the passive rate of heat exchange from the thorax than body mass. In *E. tenax*, the thorax of females is of a different shape from that of males, so mass is likely to be a better predictor of passive heat exchange than thoracic width.

Large flies passively exchange heat with the environment more slowly than small flies. This is to be expected as large flies have a smaller surface area to volume ratio and a larger thermal inertia than small flies. Therefore, small flies are going to be more affected by changes in temperature in their environment than large flies. For example, when they move through temperature gradients their body temperature will change more quickly. Small flies will warm faster and so will reach a body temperature allowing flight faster than large ones. They will also be more affected by forced convective cooling in flight, which might prevent them from keeping a high enough thoracic temperature for flight at low ambient temperature. Therefore, this purely physical effect of size is going to have an important effect on the thermal balance of these eristalines (as will be seen in Chapter 6).

4.4.6 Comparison of the two species

E. pertinax, having controlled for mass and thoracic width, warm up (both when dead and live) faster and cool down (when dead) more slowly than *E. tenax*. Neither species looks more insulated than the other. In view of what has been discussed above about the effect of evaporative cooling on the warming and cooling constants, it seems likely that this difference between the species is related to evaporative cooling as well. Evaporative cooling seems to have a bigger effect on *E. tenax* than on *E. pertinax*, because warm-up is slower and cooling down faster in *E. tenax*. This is well in accordance with the findings of Chapter 3 which showed that *E. tenax* is more "leaky" than *E. pertinax*.

Moreover, the interaction between the species and thoracic width predictors is significant: the warming and cooling constants do not change at the same rate with thoracic width in the two species.

The lack of a species effect in live cooling flies could be the result of thermo/water regulation which has been demonstrated in both species and which might confound the species effect found in dead flies. There is no interaction between species and equilibrium temperature, so the relationship between warming and cooling constants and temperature (when the other factors have been controlled for) is the same for both species.

Table 4.17 shows the cooling constants of some live endothermic insects. As only a few cooling constants for *E. pertinax* which had warmed up endothermically could be obtained, the values showed for *E. pertinax* and *E. tenax* come from flies that were warmed by a lamp.
These two eristalines have cooling and warming constants that are similar to those of other endothermic species and in particular to other flies and bees. Therefore, passive heat exchange between their body and the environment should affect their thermal balance in a similar way, and this should be reflected in their activity pattern.

Table 4.17 Cooling constants of some endothermic insects

Animal	Cooling constant °C °C^{-1} min^{-1}	Source
Bees		
Euglossine bees	0.46	May (1976a)
Xylocopa capitata	0.42	Chappell (1982)
Bombus spp.	1.25	Heinrich & Heinrich (1983)
Moths		
Cuculiine moths	0.55	Heinrich 1987
Sphingids & Saturniids	0.35	Bartholomew & Epting (1975)
Flies		
E. tenax	**0.52**	**This thesis**
E. pertinax	**0.58**	**This thesis**
Tabanus lineola	0.75	May (1976a)
Cryptotylus unicolor	0.48	May (1976a)

Water evaporation has a strong effect on the passive rates of body temperature change of these flies. It results in their equilibrium temperature being lower than ambient temperature. It was demonstrated that different values for the cooling and warming constants are obtained depending on which of equilibrium or ambient temperature is used as the baseline. So, care should be taken when estimating these constants with "leaky" animals. As it seems that evaporative cooling is not actively linked to thermoregulation, it is recommended that equilibrium temperature be used in this situation.

Evaporative cooling is probably also responsible for cooling constants being higher than warming constants, and for the difference between the species. It seems that these flies are able to control their water loss to some extent. Also, small flies are more affected by changes in temperature in their environment than large ones.

Chapter 5 - Thermoregulation

5.1 Introduction

5.1.1 Investigation of thermoregulation in active insects

"Grab and stab" is the simplest method to investigate thermoregulation in free-ranging animals. The animal is caught, and its body temperature measured by the insertion of a thermocouple into the body part of interest. Body temperatures obtained are plotted against ambient temperatures at the site and time of capture. The gradient of the best fitted regression line is then compared to the isothermal line: a gradient lower than 1 is indicative of possible thermoregulation; and the smaller the gradient the better the inference of thermoregulatory abilities. Another, and usually visually easier way, to analyse this kind of data is to plot the temperature excesses against ambient temperature: a negative relationship is indicative of thermoregulation. However, such plots only suggest the presence of thermoregulatory abilities, and it is necessary to take into account other aspects of the animal's activities to confirm that thermoregulation is taking place (May 1995).

To demonstrate thermoregulation, it is necessary to show that the animal actively controls its body temperature: thermoregulation is an active process (May 1995). Indeed, Heath (1964) demonstrated that water filled beer cans could show apparent signs of thermoregulation (the gradient of the plot of can temperature against ambient temperature was lower than 1). Also, Huey et al (1977) showed that thermoregulation can be mimicked by an animal moving randomly in its habitat, for example if more of the habitat receives solar radiation at low than at high ambient temperature. The body temperature of such an animal is thus increased at low ambient temperature by the absorption of solar radiation and is closer to ambient temperature when it is warm because less heat is gained by solar radiation. In this situation, even though the animal does not take any active steps to control its body temperature, it appears to thermoregulate.

It is therefore essential to relate apparent thermoregulation to the activities of an animal.

Another essential point to consider in such studies is the validity of the methodology used. As mentioned above, "grab and stab" is the method commonly used in the field to investigate thermoregulation in insects. Stone and Willmer (1989b) pointed out that some insects warm up endothermically when caught: thoracic temperature is then an overestimate of the true thoracic temperature in flight, especially at low ambient temperature. However, although this method might therefore give rise to some false claims of thermoregulation, they concluded that it is still the most appropriate to use in the field, if conducted with care. In the present study, to check the validity of the "grab and stab" method for *E. tenax* and *E. pertinax*, flies were attached to a very fine thermocouple (implanted in the thorax), which allowed thoracic temperature to be recorded continuously but did not restrain the flies, which could fly "freely" (they were only restricted in the direction of their flight as they had to fly in a circle). Stone and Willmer (1989b), in their assessment of the accuracy of the "grab and stab" method, used tethered bees to determine stable flight temperature (SFT): the stable thoracic temperature at which an insect flies. Thus, the bees were not affected by as much convective cooling as in real flight. Also, there is a possibility that, as they were supported, they did not "fly" with the same power as when free. In the present study, SFT was determined as close to free flight as possible, and the data were compared with the data from the "grab and stab experiment" in the laboratory.

Having ascertained that an insect does thermoregulate, it is important to know the animal's activities so as to distinguish between behavioural and physiological thermoregulation and to conduct laboratory experiments in order to identify the physiological processes involved. For example, an elevated thoracic temperature can be the result of basking, of endothermic warm-up, or both. In the present work, experiments tried to ascertain if the flies can warm up endothermically, and also investigated the thermoregulatory abilities of these insects in laboratory conditions where behavioural thermoregulation could not be operating. The flies were sampled in the field while flying, feeding or hovering and the data were compared with "grab and stab" experiments which were carried out in a controlled temperature room with no radiant

heat source and no possibility of behavioural thermoregulation. In this situation, the flies could only rely on endogenously produced heat and on physiological thermoregulation, so that the extent of physiological thermoregulation could be determined. However, it remains difficult to distinguish between physiological and behavioural processes in free-ranging animals as they are likely to use both, depending on the particular conditions faced.

Another physiological process that could be used by these flies to thermoregulate, and which is described below, is the use of haemolymph transfer from thorax to abdomen when the insect needs to cool down.

5.1.2 Haemolymph shunting - Evidence for regulation of heat flow to the abdomen in insects

When haemolymph shunting is not in operation, abdominal temperature is determined by passive heat conduction from the thorax. Thus, abdominal temperature excess is a constant proportion of thoracic temperature excess (Heinrich 1980a, 1980b; Baird 1986). A plot of the ratio of abdominal temperature excess to thoracic temperature excess (T_{abex}/T_{thex}) against ambient temperature shows that (T_{abex}/T_{thex}) is independent of ambient temperature (slope of the regression line not different from zero). When haemolymph shunting is in operation, the difference between thoracic and ambient temperature is reduced (increased heat loss from the thorax at high temperature) and the difference between abdomen and ambient temperature is raised (increased heat gain from the thorax at high ambient temperature): the proportion of abdominal temperature excess to thoracic temperature excess rises. The plot of (T_{abex}/T_{thex}) on ambient temperature has a positive slope (Baird 1986).

However, such an indication of haemolymph transfer might be hard to detect if the individuals shunt haemolymph to the abdomen at different critical ambient temperatures. The risk of overheating depends on ambient temperature, but also on the size of the insect, possibly on its reflectance, as well as its flying speed. Thus, individuals will use haemolymph shunting at various ambient temperatures depending on the particular conditions. In addition, when body temperatures are measured in the field, the thermal conditions are not necessarily stressful. The insects

might use haemolymph shunting in extreme conditions only. Such conditions are not frequently encountered in the field in Britain.

Thus, a more direct way to assess the use of haemolymph shunting is required. This involves measuring thoracic and abdominal temperatures simultaneously before, during, and after flight. When hot haemolymph is transferred to the abdomen, abdominal temperature increases and remains high.

Therefore, various aspects of thermoregulation by *E. tenax* and *E. pertinax* in relation to flight are investigated in this chapter. The endothermic abilities of these flies are also examined. In addition, the validity of the "grab and stab" method for these eristalines is checked.

5.2 Materials and Methods

All the flies used were caught in the wild and were kept as described in Chapter 4.

5.2.1 Endothermy and voluntary flight temperature

The technique for tethered flight was described in Chapter 4.

After a fly had been cooled down, it was transferred to the tank and allowed to warm up. Some flies warmed up (endothermically) and "flew" readily; others had to be stimulated by gently tapping the abdomen with a pair of forceps.

Endothermic warm-up rates (EWR) and voluntary flight temperature (VFT) were also determined in flies that were allowed to fly "freely" (see below). Endothermic warm-up rates were calculated when thoracic temperatures equalled equilibrium temperatures to eliminate any effect of passive heat exchange. In the few cases where the rates had to be estimated at another thoracic temperature, they were corrected for passive heat exchange. Voluntary flight temperature was taken as that temperature at which the fly took off the first time ("free" flight) or showed signs of trying to fly (wings beating in tethered flight). VFT was not recorded if the fly landed (or stopped beating its wings and was given the Styrofoam sphere back) and took off again without cooling down to equilibrium temperature. If several trials were carried out, or if a fly

cooled down to its equilibrium temperature before warming up and taking off again, a mean of the various VFTs has been used. Usually, the flight attempts in tethered flight were of short duration; only on very few occasions did the flies sustain flight for more than a few seconds.

5.2.2 "Grab and stab"

A/ In the field

The flies were caught with a hand net while in flight (or just after landing) or while feeding. The fly was held in a pocket of netting against a Styrofoam platform in the shade. A 40 gauge hand-made copper-constantan thermocouple mounted in a hypodermic needle and connected to a Portec thermometer (P9005, Portec Instruments Ltd, UK) was inserted in turn in the thorax and in the abdomen of the flies (the order was switched for alternate flies). Both measurements were completed within 10 s (data with longer handling times were discarded). Ambient temperature was measured immediately after this, as close as possible to the site of capture using a shaded HMI 31 thermometer and humidity probe (Vaisala Ltd, UK). Relative humidity (RH), solar radiation (with a hand held LX-101 Lux meter, Lutron, UK) and wind speed (using a hand held anemometer, Testovent 4000, Testoterm Ltd, UK) were also recorded. The flies were then weighed using a microbalance (Unwin 1980) and released.

B/ In the laboratory

The experiments were carried out in a controlled temperature room with no window. The flies were fed before and were kept in a cool box. They were released on a wooden stool and left until they took off (some needed to be slightly stimulated by gently tapping with a pair of forceps). Once in flight, they were left to fly for about a minute (and were forced to take off again if they landed) to allow stable flight temperature to be reached. Thoracic and ambient temperatures were then recorded as described above.

5.2.3 "Free" flight

A fine 48 gauge hand-made copper-constantan thermocouple (finer than the ones used for tethered flight) was inserted in the thorax as described in Chapter 4. The thermocouple was not held in a clamp. The

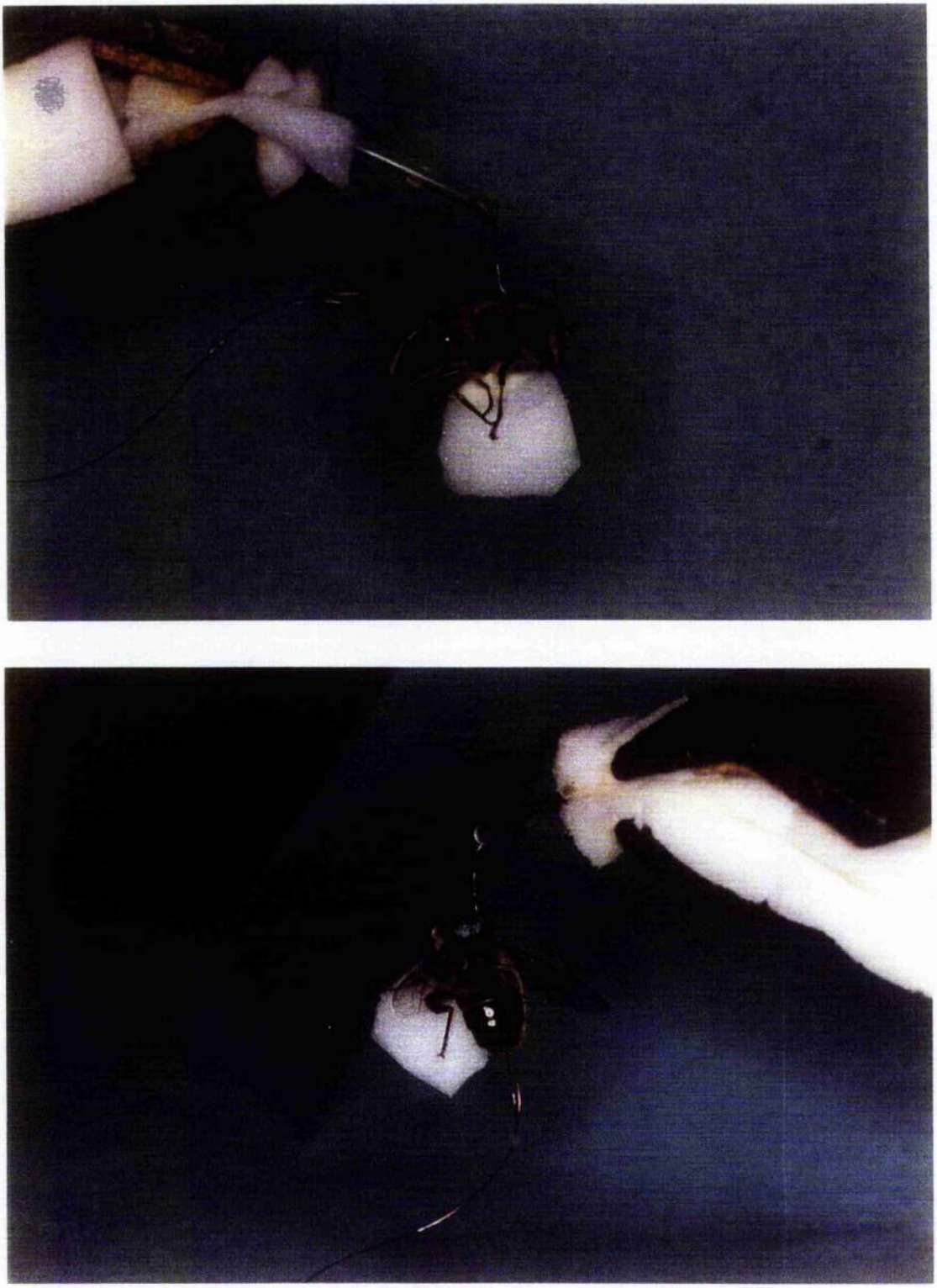


Fig 5.1 Photographs of a female *E. tenax* with thermocouples implanted in the thorax and the abdomen (for haemolymph shunting experiment)

flies were cooled in a cool box, and then released as before. During flight, the thermometer (PI 8013, Portec Instruments Ltd., UK) was held in the air to allow the fly to fly "freely" (unsupported) in a circle. Results from flies which did not produce a powerful enough flight to support themselves were discarded. Thoracic temperature was continuously recorded on a chart recorder as elsewhere. Some flies were not fed, others were either fed before or between trials of the experiment. SFT was measured from the recorded traces and was plotted against temperature as for the "grab and stab" experiments. Both equilibrium and ambient temperature were taken as baselines (cf. Chapter 4) and the results were compared.

5.2.4 Haemolymph shunting

For the haemolymph shunting experiments, 48 gauge hand-made copper-constantan thermocouples were used; one was inserted, as already described, in the thorax and another dorsally between the first and second abdominal tergites (Figure 5.1).

5.2.5 Statistical methods

The same statistical methods as described in Chapter 4 were used.

5.3 Results

5.3.1 Endothermy

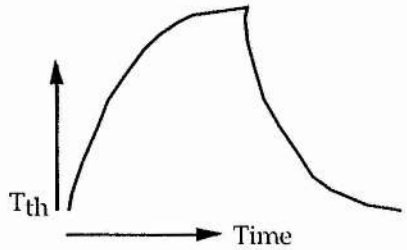
A/ Description of endothermic warm-up

Some flies started warming up endothermically as soon as they were taken out of the cooled container. Some underwent a period of passive warm-up before resorting to endothermy, others needed to be gently tapped with a pair of forceps to show any endothermic activity. During endothermic warm-up, no movement of the wings could be seen and no noise could be heard, but deep abdominal pumping movements occurred. Frequently, the flies "walked", that is, moved around the Styrofoam sphere between their legs. Not all the endothermic bouts culminated in flight attempts; some flies passively cooled down after a bout. In some cases, this was followed by further endothermic bouts (Table 5.1, Figure 5.2). Some flies also kept an elevated thoracic temperature for a considerable

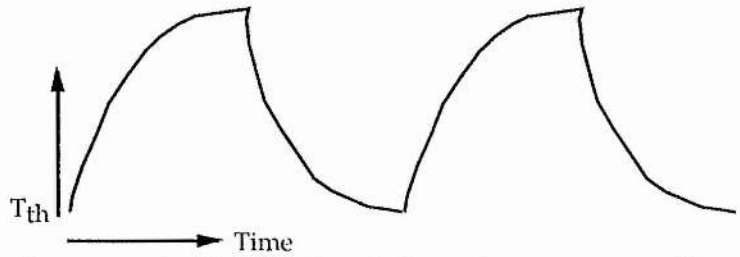
time (one fly kept a thoracic temperature excess (T_{thex}) of 8.7 °C for 23 minutes). Table 5.1 shows the endothermic patterns exhibited by a few unfed and fed *E. tenax* (similar results were obtained for *E. pertinax*) during tethered flight experiments. The four patterns (a, b, c, and d) are shown on Figure 5.2 and represent:

- a: a single endothermic warm-up bout followed by passive cooling down,
- -b: successive bouts of endothermic warm-up with passive cooling down at a thoracic temperature close equilibrium temperature in between,
- c: successive bouts of endothermic warm-up with passive cooling down in between but not as low as equilibrium temperature,
- d: endothermic warm-up with maintenance of a fairly constant elevated thoracic temperature.

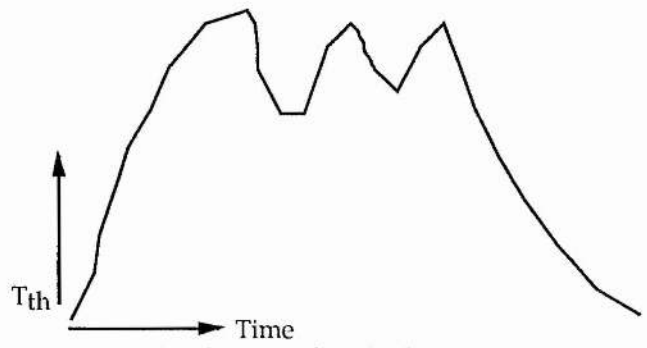
From Table 5.1 it seems that the repetitions and the duration of the patterns appeared to increase after feeding.



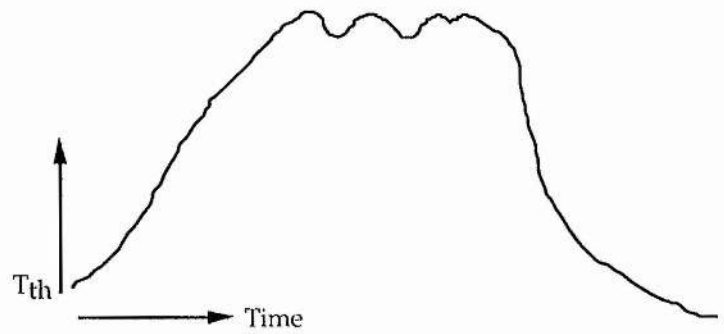
Pattern a: a single endothermic warm-up followed by passive cooling down.



Pattern b: successive bouts of endothermic warm-up with passive cooling down to a T_{th} close to T_{eq} in between.



Pattern c: successive bouts of endothermic warm-up with passive cooling down in between but not as low as T_{eq} (T_{th} remains elevated).



Pattern d: endothermic warm-up with maintenance of a fairly constant elevated T_{th}.

Fig. 5.2 Patterns of endothermy in *E. tenax* and *E. pertinax*

Table 5.1 A sample of endothermic patterns in *E. tenax*. Flies that were unfed and did not show any endothermy are not included.

Before feeding Pattern (Duration (min))	T_a (°C)	**After feeding** Pattern (Duration (min))	T_a (°C)
		c (16.0), rest (6.8), b (21.3), c (13.5), rest (4.5), b (15.3, rest (32), b (34.4), rest (interrupted)	14.7
		no endothermy	15.5
a (10.1), rest	12.8	b (39.1), rest	13.8
b [15.3, +d (5.5)], rest	14.8		
		a (7.1), rest (16.2), b (42.0), rest (interrupted)	12.5
		b[114.4, +c (17.4-9.2-12.3)], rest	15.5
		a (11.3), rest	13.7
		a (13.7), rest (4.7), c (40.3), rest (4.0), c (50.8), rest (8.6), c (24.0), rest (interrupted)	13.4
a (3.5), rest	13.1	c (5.1), rest	14.0
a (2.8), rest	12.9	a (6.7). rest	11.8
no endothermy	13.4	c (9.5), rest	13.4
c (5.7), rest	14.8	a (4.7), rest	13.6
no endothermy	13.5	no endothermy	13.5
a (8.9), rest (26.3), a (1.9), rest	15.6	a (12.4), rest (4.3), c (8.4), rest	14.5
c (9.5), rest	14.4	no endothermy	14.6
no endothermy	15.3	a (5.3), rest (3.3), c (8.4), rest (1.7), c (9.1), rest (1.2), c (50.8), rest (4.6), c (30.8), rest (5.8), c (26.6), rest (3.5), c (22.6), rest (3.2),c [55.3, +d (18.0)], rest (interrupted)	15.3
		no endothermy	16.4
a (4.5), rest	12.1	b (31), rest	11.3
		d (22.7), rest (6.4), c (5.9), rest (13.8), d (9.3), rest (interrupted)	13.2
a (6.0), rest	13.3	c (11.7), rest	13.3

Endothermic warm-up traces were curvilinear. Figure 5.3 shows a typical warm-up trace for *E. tenax* or *E. pertinax*. As thoracic temperature increased, the rate of warm-up decreased.

The maximum thoracic temperature excess (T_{ex}) achieved by *E. tenax* was 14.2 °C at an equilibrium temperature of 11 °C. *E. pertinax* reached a maximum thoracic temperature excess of 8.6 °C at an equilibrium temperature of 12 °C. It was more difficult to induce endothermic warm-up at low than at medium equilibrium temperature but, quite often, at high equilibrium temperature, the flies did not require the use of endothermy as a thoracic temperature high enough to support flight could be reached by passive warming to the ambient temperature.

B/ Endothermic warm-up in *E. tenax*

Covariance analyses were carried out to see the effect of size, thoracic temperature, sex and season on the rate of endothermic warm-up in unfed and fed flies. The logarithm of EWR had to be used to make the data normal. Mass was not a significant factor in determining the rate of endothermic warm-up but thoracic width was. It was therefore used throughout the analyses.

a/ Unfed flies

Table 5.2 shows that the logarithm of EWR has a positive relationship with both thoracic temperature and thoracic width. The results of the regression of the logarithm of EWR against thoracic temperature and thoracic width are shown in Figure 5.4a & b (n=30, R^2=0.50, T_{th} $p<0.001$, Th_w $p=0.005$). Therefore, unfed *E. tenax* warm up faster if their thorax is warm than if it is cold, and flies with a large thorax warm up faster than flies with a small thorax. There is no effect of sex or season on the warm-up rates.

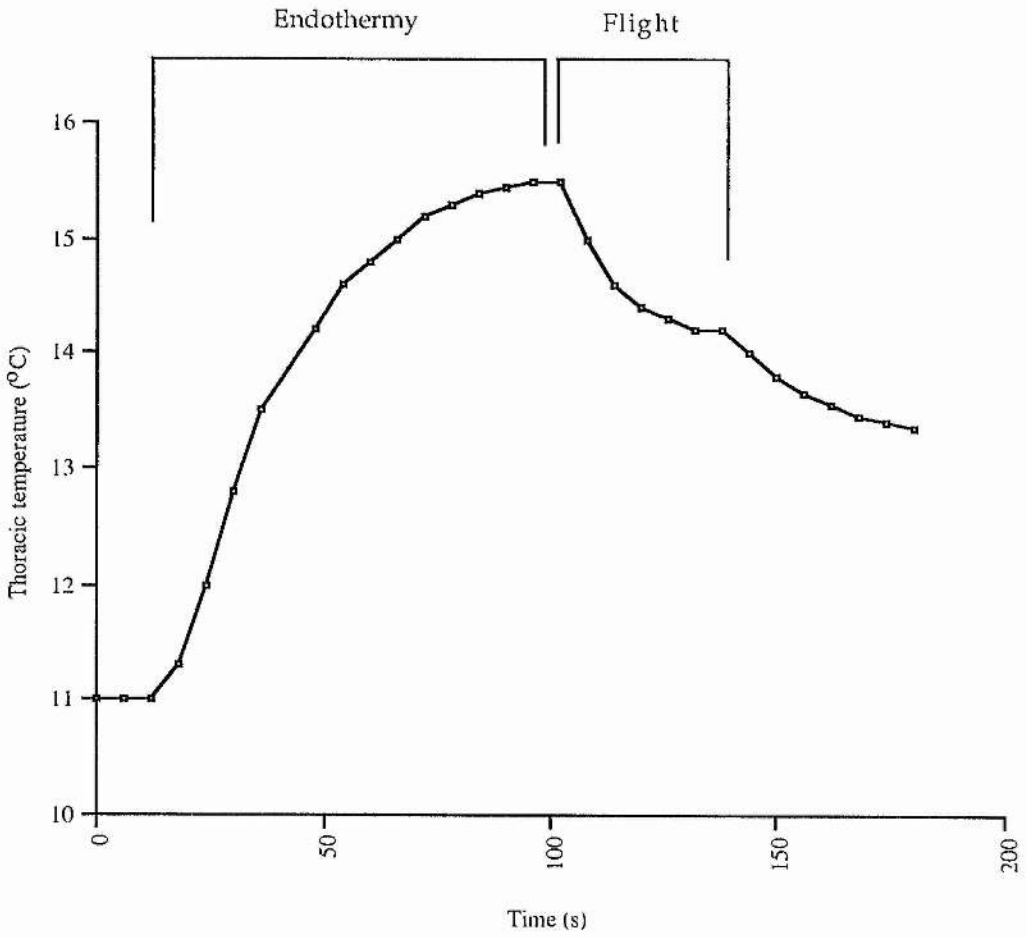
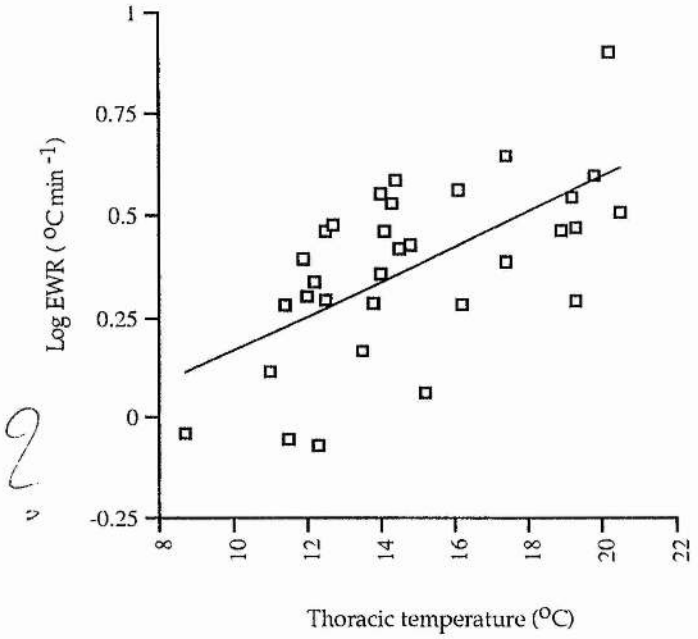


Fig. 5.3 A typical endothermic warm-up trace by *E. tenax* or *E. pertinax*

a/



b/

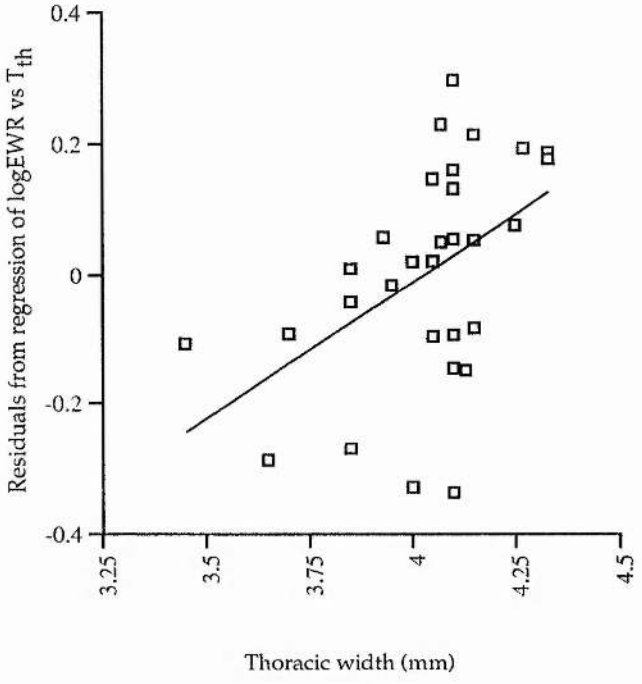


Fig. 5.4 Endothermic warm-up in unfed *E. tenax*

a/ Log endothermic warm-up rate vs thoracic temperature,
$y = 0.043x - 0.258$, $r^2 = 0.385$
b/ Residuals of LogEWR vs T_{th} against thoracic width,
$y = 0.425x - 1.712$, $r^2 = 0.231$

Table 5.2 Covariance analysis on logEWR for T_{th}, Th_w, sex and season in unfed *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{th}	1	0.598	0.532	0.532	21.25	<0.001
Th_w	1	0.024	0.191	0.191	7.65	0.011
Sex	1	0.004	0.004	0.004	0.16	0.691
Season	1	0.00003	0.00003	0.00003	0.00	0.971
Error	25	0.626	0.626	0.025		
Total	29	1.252				

b/ Fed flies

In fed flies, the logarithm of EWR has a positive relationship with thoracic temperature, but no relationship with thoracic width (Table 5.3). Thus again, flies with a warm thorax warm up faster than flies with a cold thorax. However, after feeding, the relationship between warm up rate and thoracic width disappears. There is no effect of sex or season on the warm-up rates.

Table 5.3 Covariance analysis on logEWR for T_{th}, Th_w, sex and season in fed *E. tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{th}	1	1.189	0.803	0.803	42.48	<0.001
Th_w	1	0.027	0.059	0.059	3.12	0.087
Sex	1	0.0004	0.0046	0.0046	0.24	0.626
Season	1	0.032	0.032	0.032	1.70	0.202
Error	30	0.567	0.567	0.019		
Total	34	1.816				

c/ Effect of feeding state

The following analysis was carried out in order to check if feeding has an effect on the endothermic warm-up rate. In this analysis, a fly was either unfed or fed, but did not contribute to both categories.

A covariance analysis (Table 5.4) of the effect of thoracic temperature, thoracic width, sex, season, feeding state and the interactions

between feeding state and thoracic width and between feeding state and thoracic temperature on the logarithm of EWR shows a significant interaction between feeding state and thoracic width. This reflects the loss of the relationship between the logarithm of EWR and thoracic width after feeding (as described above). Feeding state is also a significant predictor of the logarithm of EWR, and the fitted means indicate that after feeding, *E. tenax* flies warm up more slowly than before feeding. As above, there is no effect of sex or season on the warm-up rates. The regression lines between thoracic temperature and the logarithm of EWR have the same gradient in unfed and fed flies (interaction between feeding state and thoracic width not significant).

Table 5.4 Covariance analysis on logEWR for T_{th}, Th_w, sex, feeding state and the interactions between feeding state and Th_w and between feeding state and T_{th} season in E. *tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{th}	1	1.500	1.296	1.296	68.48	<0.001
Th_w	1	0.019	0.015	0.015	0.79	0.378
Sex	1	0.00001	0.0058	0.0058	0.31	0.583
Season	1	0.0008	0.0195	0.0195	1.03	0.315
Feeding	1	0.022	0.258	0.258	13.61	0.001
Feed.*Th_w	1	0.245	0.271	0.271	14.31	<0.001
Feed.*T_{th}	1	0.026	0.026	0.026	1.38	0.245
Error	48	0.908	0.908	0.019		
Total	55	2.721				

Fitted means for logEWR

	Mean (°C/min)	Stdev (°C/min)
Before feeding	0.43 (EWR=2.69)	0.030
After feeding	0.38 (EWR=2.40)	0.030

C/ Endothermic warm-up in *E. pertinax*

The same covariance analyses as for *E. tenax* were carried out. Mass and thoracic width show similar relationships with the logarithm of EWR so, for clarity and to make the data comparable with those from *E. tenax*, only the analyses carried out with thoracic width as the size factor are shown.

a/ Unfed flies

The regressions of the logarithm of EWR on thoracic temperature and on thoracic width are shown in Figure 5.5a & b. Table 5.5 shows that the logarithm of EWR has a positive relationship with thoracic temperature but a negative relationship with thoracic width (n=7, R^2=0.92, T_{th} p=0.009, Th_w p=0.013). Therefore, unfed *E. pertinax* with a warm thorax warm up faster than those with a cold thorax. Surprisingly, flies with a large thorax have smaller endothermic warm-up rates than flies with a small thorax. Sex has no effect on EWR.

Table 5.5 Covariance analysis on logEWR for T_{th}, Th_w and sex in unfed *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{th}	1	0.314	0.230	0.230	16.26	0.027
Th_w	1	0.196	0.195	0.195	13.76	0.034
Sex	1	0.0009	0.0009	0.0009	0.06	0.815
Error	3	0.042	0.042	0.014		
Total	6	0.554				

b/ Fed flies

In fed flies (Table 5.6), the logarithm of EWR still has a positive relationship with thoracic temperature (i.e. the warm-up rate of fed *E. pertinax* increases with thoracic temperature), but it is not correlated with thoracic width. The size of the thorax does not have an effect on the endothermic warm-up rate; nor has sex.

Table 5.6 Covariance analysis on logEWR for T_{th}, Th_w and sex in fed *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{th}	1	0.374	0.364	0.364	16.39	0.005
Th_w	1	0.0003	0.00001	0.00001	0.00	0.985
Sex	1	0.008	0.008	0.008	0.37	0.563
Error	7	0.156	0.156	0.022		
Total	10	0.539				

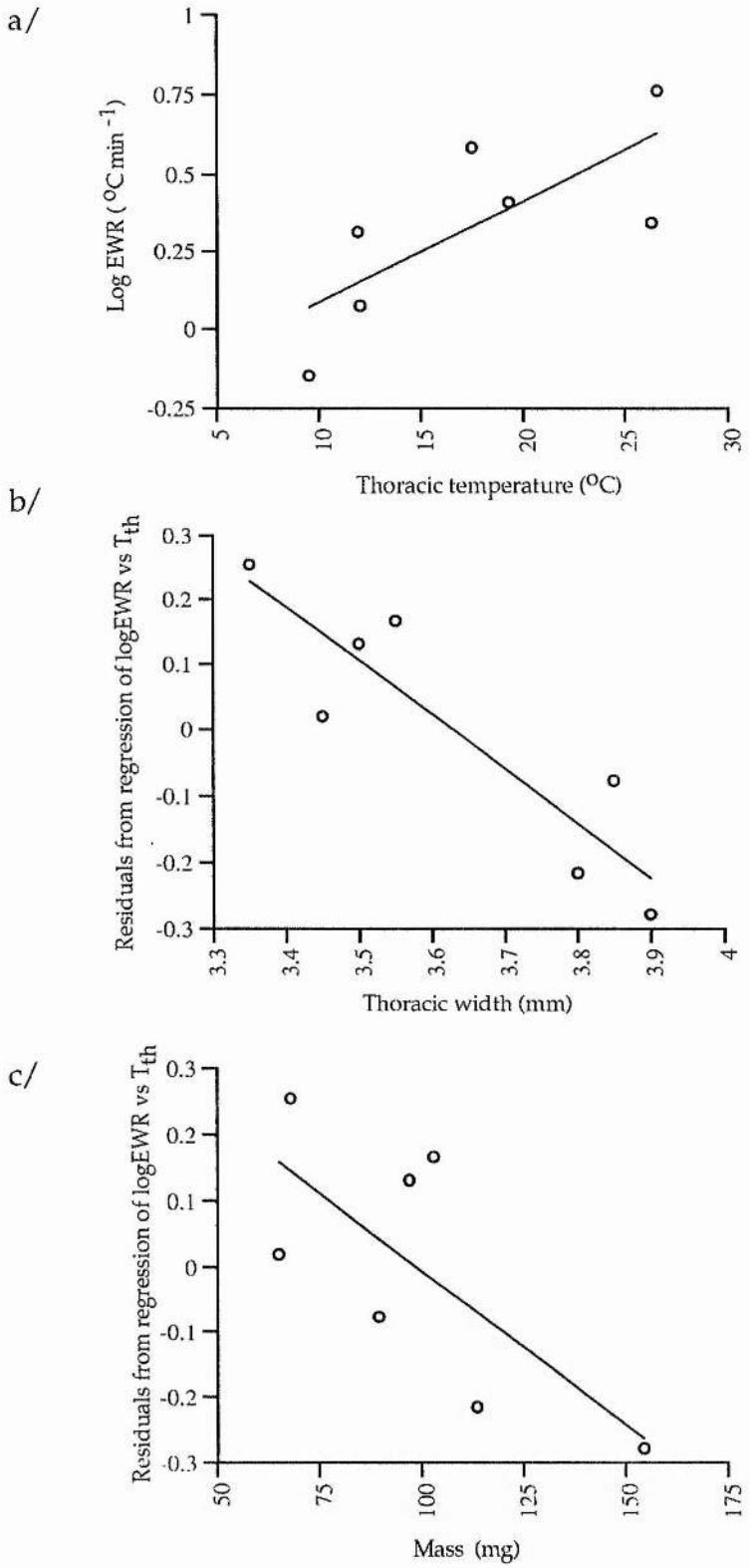


Fig. 5.5 Endothermic warm-up in unfed *E. pertinax*

a/ LogEWRvs T_{th}, $y = 0.033x - 0.246$, $r^2 = 0.567$

b/ Residuals of LogEWR vs T_{th} against Th_W, $y = -0.823x + 2.987$, $r^2 = 0.803$

c/ Residuals of LogEWR vs T_{th} against mass, $y = -0.005x + 0.465$ $r^2 = 0.511$

c/ Effect of feeding state

A covariance analysis was carried out on the logarithm of EWR for thoracic width, thoracic temperature, sex, feeding state and the interactions of feeding state with thoracic width and with thoracic temperature (Table 5.7) to check for an effect of feeding on the endothermic warm-up rate. The small sample size can probably account for the absence of a significant interaction between feeding state and thoracic width which was expected from the separate analyses for unfed and fed flies. In *E. pertinax*, feeding state has no effect on the logarithm of EWR. Excluding the interactions between feeding state and thoracic width and feeding state and thoracic temperature from the model did not change the results.

Table 5.7 Covariance analysis on logEWR for T_{th}, Th_w, sex, feeding state and the interactions between feeding state with Th_w and T_{th} in *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{th}	1	0.683	0.584	0.584	16.53	0.002
Th_w	1	0.0002	0.004	0.004	0.11	0.750
Sex	1	0.011	0.0097	0.0097	0.27	0.611
Feeding	1	0.021	0.002	0.002	0.06	0.816
Feed.*Th_w	1	0.001	0.001	0.001	0.03	0.866
Feed.*T_{th}	1	0.004	0.005	0.005	0.15	0.706
Error	11	0.389	0.89	0.035		
Total	17	1.108				

D/ The two species compared

Table 5.8 shows the results of a covariance analysis on the logarithm of EWR for thoracic temperature, thoracic width, mass, species and the interactions between species and thoracic temperature, between species and thoracic width, and between species and mass. Mass has been included in this analysis because it is a significant predictor of the logarithm of EWR in *E. pertinax* and to eliminate any mass effect between the species. Sex, feeding state and the interaction between feeding state and species were included in another analysis, but were not significant and were omitted from the present analysis. This analysis confirms that

the logarithm of EWR has a positive relationship with thoracic temperature. Thoracic width and mass do not have an effect on the logarithm of EWR, but this might stem from the fact that the species show different relationships between these factors (*E. tenax* shows a positive relationship whereas *E. pertinax* has a negative relationship); this is confirmed by the significant interaction between species and thoracic width (an interaction between species and mass could not be demonstrated though, probably because the difference between the species is not so important as *E. tenax* does not show a relationship between the logarithm of EWR and mass).
Overall then, species is a significant predictor of the logarithm of EWR. Once the other factors have been controlled for, *E. tenax* have larger endothermic warm-up rates than *E. pertinax*, i. e. they warm up faster.

Table 5.8 Covariance analysis on logEWR for T_{th}, Th_w, mass, species and the interactions between species and T_{th}, between species and Th_w, and between species and mass for *E. tenax* and *E. pertinax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{th}	1	2.068	1.937	1.937	86.94	<0.001
Th_w	1	0.030	0.044	0.044	1.98	0.164
Mass	1	0.0005	0.0067	0.00067	0.30	0.584
Species	1	0.072	0.122	0.122	5.47	0.022
Species*T_{th}	1	0.032	0.044	0.044	2.00	0.162
Species*Th_w	1	0.165	0.094	0.094	4.20	0.044
Species*m	1	0.005	0.005	0.005	0.23	0.630
Error	66	1.470	1.470	0.022		
Total	73	3.84				

Fitted means for logEWR

	Mean (°C/min)	Stdev (°C/min)
E. tenax	0.41 (EWR=2.56)	0.022
E. pertinax	0.16 (EWR=1.43)	0.078

E/ Summary

In this section, it has been demonstrated that both species can warm up endothermically. When thoracic temperature, thoracic width and mass are controlled for, *E. tenax* has better endothermic abilities than *E. pertinax*.

Large, unfed flies warm up faster than small, unfed flies, but this relationship disappears after feeding. Also warm flies have higher endothermic warm-up rates than cool ones. Feeding seems to induce more repetitions and a longer duration of endothermic bouts.

5.3.2 Voluntary Flight Temperature

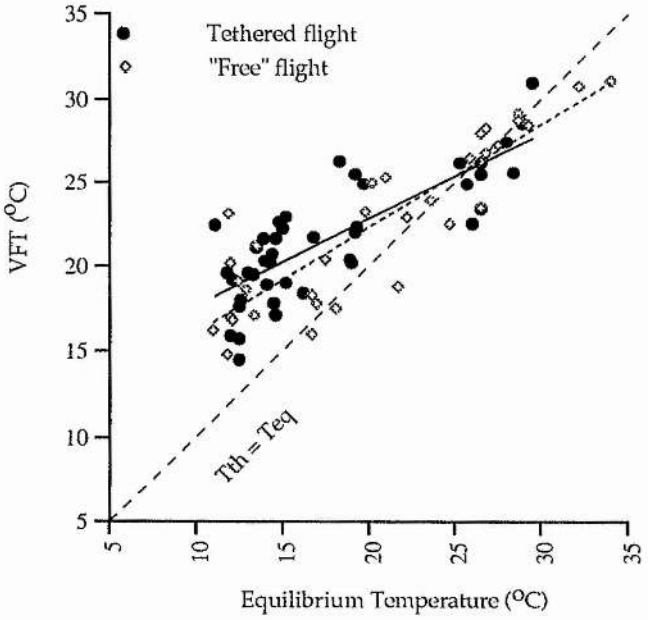
VFT could show a relationship with either mass or thoracic width (or both). Thoracic width was tested, but was not a significant predictor of VFT. Therefore, the size factor shown in all these analyses is mass.

For the reasons stated above, all the analyses were done with equilibrium temperature as the baseline; as a check, the same analyses were carried out with ambient temperature and gave very similar results.

A/ *E. tenax*

An analysis of covariance looking at the effect of equilibrium temperature, mass, sex, season, type of flight (tethered or "free") and feeding state on VFT was carried out (Table 5.9). Figure 5.6a & b shows the regressions for tethered and "free" flight of VFT against equilibrium temperature (tethered flight: n=42, R^2=0.66, p<0.001; "free" flight: n=35, R^2=0.79, p<0.001) and of VFT_{ex} against equilibrium temperature (tethered flight: n=42, R^2=0.63, p<0.001; "free" flight: n=35, R^2=0.58, p<0.001). VFT has a positive relationship with equilibrium temperature, and VFT_{ex} has a negative relationship with equilibrium temperature. As explained in the introduction, a significant relationship between VFT_{ex} and temperature indicates that VFT does not follow the isothermal line. The other factors do not have any significant effect. Therefore, VFT increases with temperature, but the difference between voluntary flight temperature and equilibrium temperature decreases as equilibrium temperature increases: VFT_{ex} is larger at low than at high temperature. Thus, *E. tenax* do not keep their take off temperature constant over the range of temperatures investigated (11 to 34°C), but VFT is regulated to some extent as the regression line of VFT against temperature is not parallel to the isothermal line. There is no difference between flight type, and mass, sex, season and feeding state do not have an effect on VFT. Separate analyses for unfed and fed flies did not show any influence of mass, sex or season on VFT either.

a/



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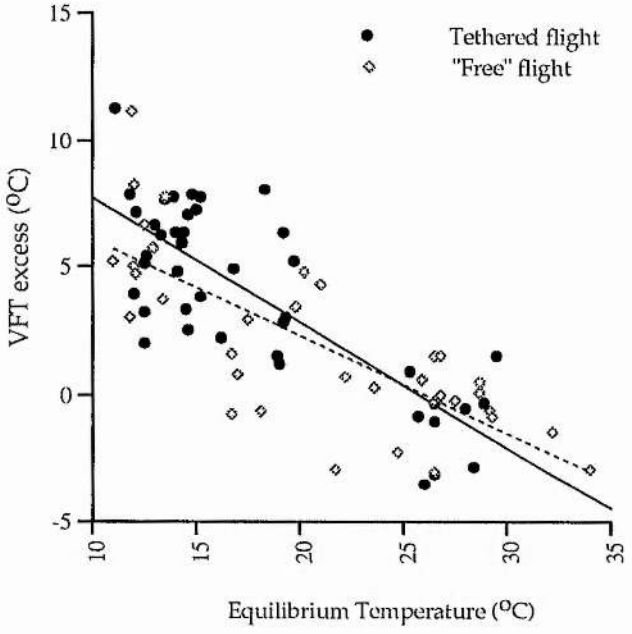


Fig. 5.6 Voluntary flight temperature in *E. tenax*

a/ VFT versus T_{eq}: tethered flight, y = 0.515x + 12.508, r^2 = 0.659;
"free" flight, y = 0.624x + 9.810, r^2 = 0.794
b/VFT excess versus T_{eq}: tethered flight, y = -0.485x + 12.508, r^2 = 0.632;
"free" flight, y = -0.376x + 9.810, r^2 = 0.583

Table 5.9 Covariance analysis on VFT for T_{eq}, mass, sex, season, feeding state and flight type in *E. tenax*

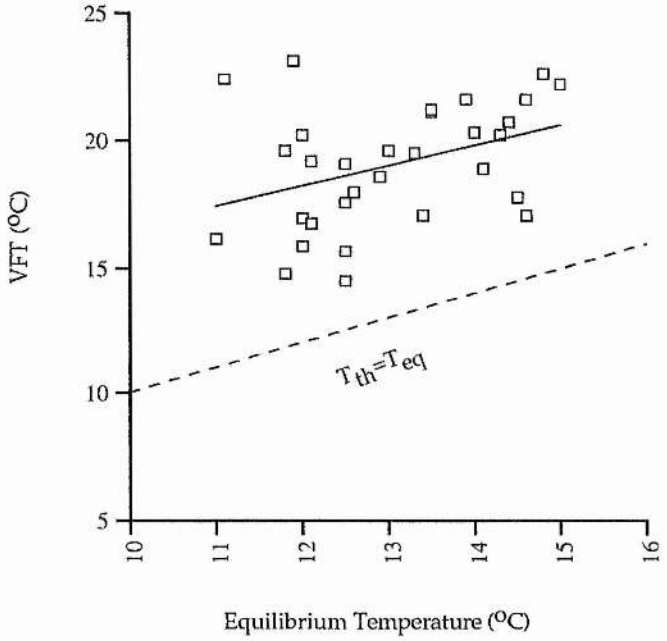
Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	1003.97	926.65	926.65	199.28	<0.001
Mass	1	22.04	10.86	10.86	2.33	0.131
Sex	1	5.61	3.15	3.15	0.68	0.413
Season	1	0.11	0.21	0.21	0.05	0.831
Feeding	1	5.78	4.34	4.34	0.93	0.337
Flight type	1	7.08	7.08	7.08	1.52	0.221
Error	70	325.50	325.50	4.65		
Total	76	1370.09				

Flies only need to warm up before flight at low ambient temperature, so it is likely that if a mass effect on VFT is present it will be at low temperature. Thus, equilibrium temperature was restricted to a maximum of 15 °C to investigate the effect of mass (flight types lumped together)(Table 5.10). The other factors (sex, season and feeding state) were not significant and have been omitted. Figure 5.7a & b shows the positive relationships between VFT and equilibrium temperature and mass, with equilibrium temperature being limited to a maximum of 15 °C ($n=31$, $R^2=0.26$, T_{eq} $p=0.048$, mass $p=0.042$). Therefore, at low temperature, when they need to warm up endothermically (in the laboratory) to be able to fly, heavy *E. tenax* take off at a higher temperature than light ones.

Table 5.10 VFT data analysis of covariance or T_{eq} (up to maximum of 15 °C) for T_{eq} and mass in E. *tenax*

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	24.038	19.073	19.073	4.26	0.048
Mass	1	20.280	20.280	20.280	4.53	0.042
Error	28	125.315	125.315	4.476		
Total	30	169.634				

a/



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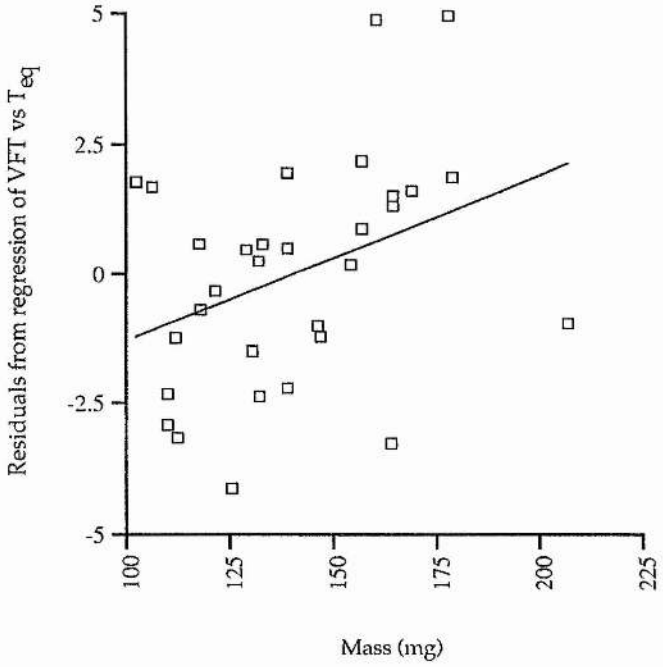


Fig. 5.7 Voluntary flight temperature in *E. tenax* (maximum T_{eq} of 15 °C)

a/ VFT versus T_{eq}; $y = 0.780x + 8.864$, $r^2 = 0.142$

b/ Residuals from regression of VFT vs T_{eq} against mass,

$y = 0.032x - 4.545$, $r^2 = 0.138$

B/ *E. pertinax*

The analysis of covariance shown in Table 5.11 reveals a positive relationship between VFT and equilibrium temperature. Flight type is also a significant predictor of VFT once the other factors have been controlled for, but mass, sex and feeding state are not (equilibrium temperature could not be restricted due to the small sample size for each flight type). Figure 5.8a & b shows the regression of VFT against equilibrium temperature for both experimental procedures (tethered flight: n=11, R^2=0.82, p<0.001; "free" flight: n=15, R^2=0.96, p<0.001) and of VFT_{ex} against equilibrium temperature (tethered flight: n=11, R^2=065, p=0.003; "free" flight: n=15, R^2=0.60, p<0.001). VFT increases with temperature and VFT_{ex} decreases with temperature: at low temperature *E. pertinax* need to reach a higher temperature relative to equilibrium temperature than at high temperature before they can fly. VFT is not kept constant over the range of temperatures investigated (10.7-33.0 °C): *E. pertinax* can take off at various thoracic temperatures. However, the regression line of VFT against temperature does not follow the isothermal line: VFT is regulated. Voluntary flight temperature is higher in the tethered flight experiment than in the "free" flight experiment.

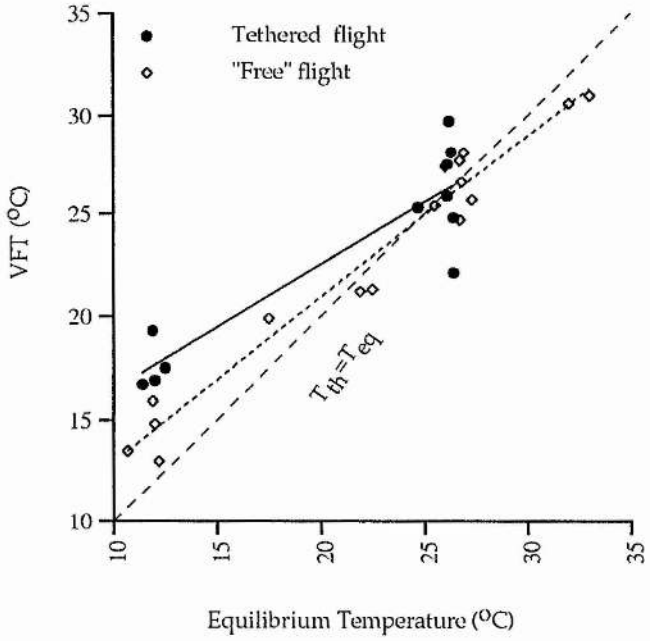
Table 5.11 VFT data analysis of covariance on VFT for T_{eq}, mass, sex, feeding state and flight type for *E. pertinax* ; all factors included

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	686.17	514.17	514.17	167.37	<0.001
Mass	1	4.71	0.31	0.31	0.10	0.754
Sex	1	0.31	0.09	0.09	0.03	0.869
Feeding	1	0.94	0.82	0.82	0.27	0.611
Flight type	1	6.83	16.45	16.45	5.35	0.031
Flight type*T_{eq}	1	11.10	11.10	11.10	3.61	0.072
Error	20	61.44	61.44	3.07		
Total	26	771.49				

Fitted means for VFT

	Mean (°C)	Stdev (°C)
Tethered flight	23.67	0.63
"Free" flight	22.31	0.60

a/



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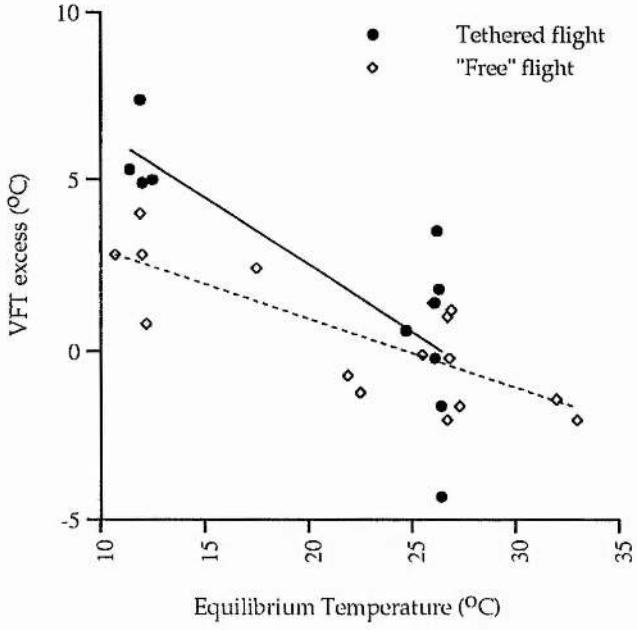


Fig. 5.8 Voluntary flight temperature in *E. pertinax*

a/ VFT versus T_{eq}: tetherd flight, $y = 0.609x + 10.343$, $r^2 = 0.819$;
"free" flight, $y = 0.801x + 4.928$, $r^2 = 0.960$,
b/VFT excess versus T_{eq}: tethered flight, $y = -0.391x + 10.343$, $r^2 = 0.651$;
"free" flight, $y = -0.199x + 4.928$, $r^2 = 0.599$

C/ Comparison of the two species

As the type of flight is a significant predictor of VFT in *E. pertinax*, flight types had to be separated. It was decided to compare the species using data from the "free" flight experiment, as these are more likely to reflect the VFT encountered in wild flies (Table 5.12). Again, sex and feeding state were non-significant predictors of VFT and were omitted from the analysis shown here. Mass was kept in the analysis to account for the species size difference. Figure 5.9a & b shows the regression of VFT against equilibrium temperature for both species ("free "flight) (*E. tenax*: n=35, R^2=0.79, p<0.001; *E. pertinax*: n=15, R^2=0.96, p<0.001) and of VFT_{ex} against equilibrium temperature (*E. tenax*: n=35, R^2=0.58, p<0.001; *E. pertinax*: n=15, R^2=0.60, p<0.001). The gradient of the regression of VFT against equilibrium temperature (and of VFT_{ex} against equilibrium temperature) differ between the species (the interaction between species and equilibrium temperature is almost significant). This seems to be due to the fact that, once the other factors have been controlled for, *E. tenax* has higher VFTs than *E. pertinax* at low equilibrium temperature (i.e. species is a significant predictor of VFT).

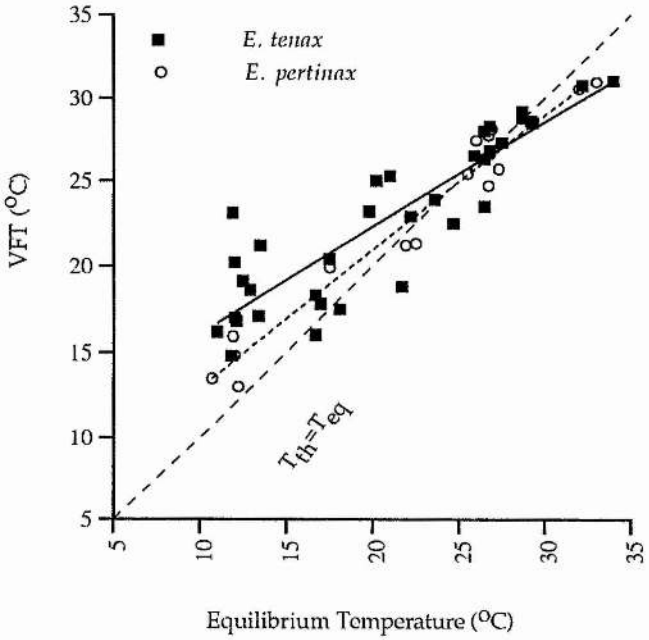
Table 5.12 VFT data analysis of covariance on VFT for T_{eq}, mass and species (comparison of E. *pertinax* and *E. tenax*)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	1127.60	1067.61	1067.61	271.59	<0.001
Mass	1	15.69	5.69	5.69	1.45	0.235
Species	1	2.66	17.48	17.78	4.52	0.039
Species*T_{eq}	1	15.17	15.15	15.15	3.86	0.056
Error	46	180.85	180.85	3.93		
Total	50	1341.97				

Fitted means for VFT

	Mean (°C)	Stdev (°C)
E. tenax	23.03	0.3505
E. pertinax	22.32	0.5501

a/



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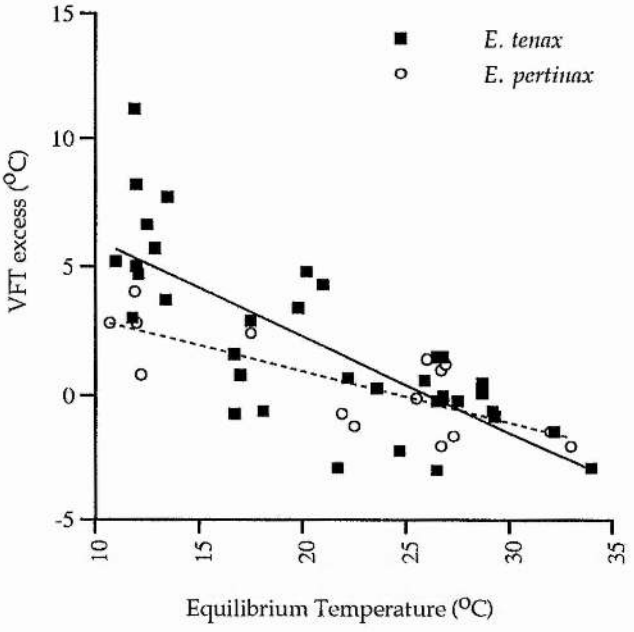


Fig. 5.9 Voluntary flight temperature ("free" flight), *E. tenax* and *E. pertinax*

a/ VFT versus T_{eq}: *E. tenax*, $y = 0.624x + 9.810$, $r^2 = 0.794$;
E. pertinax, $y = 0.801x + 4.928$, $r^2 = 0.960$
b/ VFT_{ex} vs T_{eq}: *E. tenax*, $y = -0.376x + 9.810$, $r^2 = 0.583$;
E. pertinax, $y = -0.199x + 4.928$, $r^2 = 0.599$

D/ Summary

The gradient of the regression line of VFT against temperature does not follow the isothermal line: this suggests than VFT is regulated. However VFT is not maintained constant over the temperature ranges of this study: it increases with temperature. When temperature and mass are controlled for, *E. tenax* has higher voluntary flight temperatures than *E. pertinax*. At low temperature, when they need to warm up endothermically prior to flight, large *E. tenax* have higher voluntary flight temperatures than small ones. However, no effect of mass could be detected at high temperatures or for *E. pertinax*.

5.3.3 Thermoregulation in flight

A/ "Grab and stab" in the laboratory

a/ *E. tenax*

E. tenax was very reluctant to fly at ambient temperatures below 8 °C (even when stimulated). The flies took off and flew at ambient temperatures ranging from 8 to 29°C. Both thoracic and abdominal temperatures have a positive relationship with ambient temperature (regressions: T_{th}, n=17, R^2=0.975, p<0.001; T_{ab}, n=17, R^2=0.99, p<0.001). Figure 5.10a & b shows plots of thoracic temperature, abdominal temperature, thoracic temperature excess (T_{thex}) and abdominal temperature excess (T_{abex}) against ambient temperature. No difference was observed between the sexes, and mass or thoracic width were not significant predictors of thoracic temperature in flight (covariance analysis not shown here). The gradients of the regression lines for both thoracic and abdominal temperature excesses on ambient temperature are different from zero (regressions: T_{thex}, n=17, R^2=0.62, p<0.001; T_{abex}, n=17, R^2=0.52, p<0.001). In the laboratory, *E. tenax* do not keep a constant thoracic or abdominal temperature in flight over this range of ambient temperatures, but the temperature excesses (both for the thorax and the abdomen) are higher at low ambient temperature than at high. Hence, thoracic and abdominal temperatures do not parallel ambient temperature (gradient smaller than 1); they are regulated. *E. tenax* achieves thoracic temperatures of around 14 °C at ambient temperatures of 8 °C in flight. This temperature excess of about 6°C, generated by endothermy alone, is reduced to about 2.5°C at 29°C.

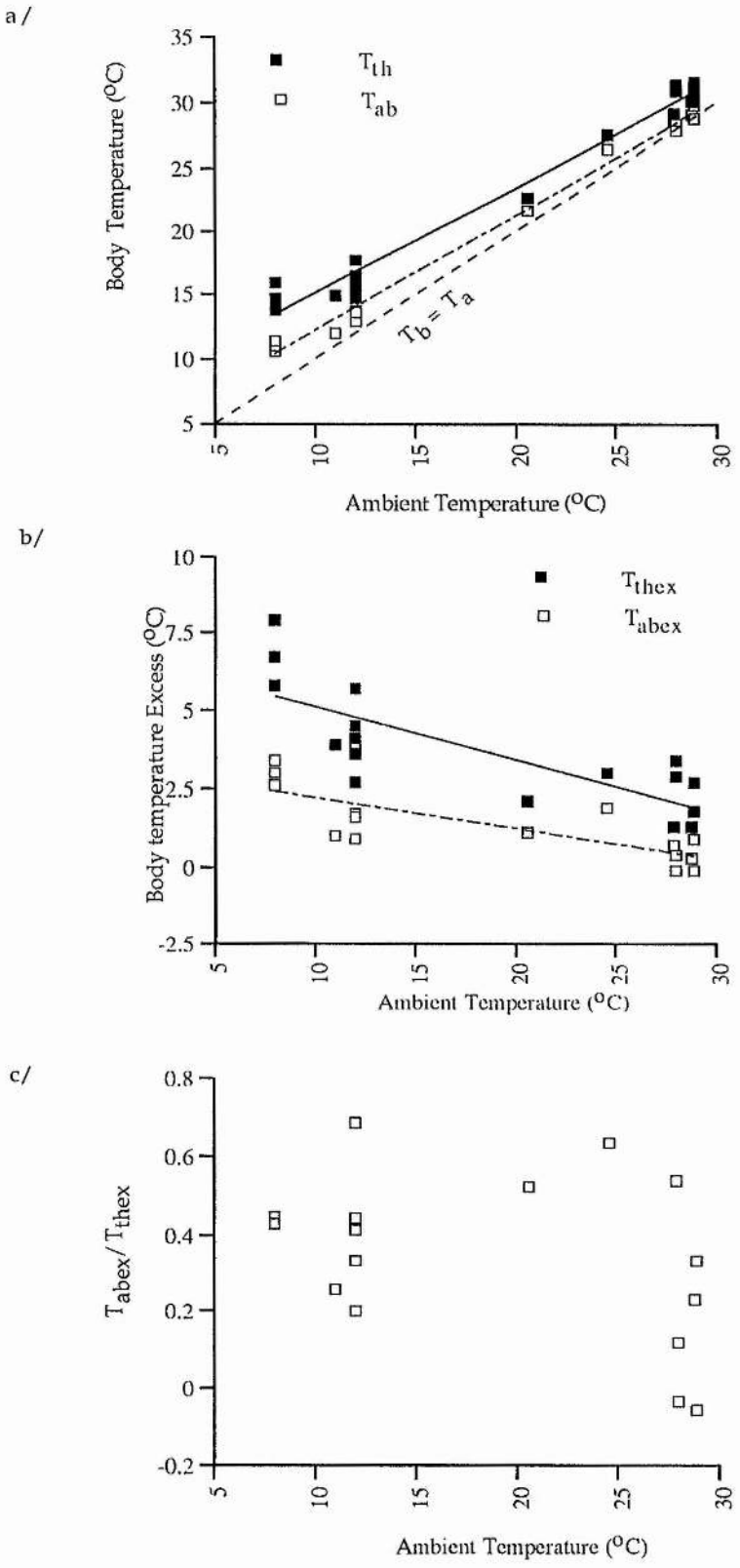


Fig. 5.10 *E. tenax* "grab and stab" in the laboratory
a/ T_{th} vs T_a, $y = 0.831x + 6.822$, $r^2 = 0.975$; T_{ab} vs T_a, $y = 0.902x + 3.206$, $r^2 = 0.989$
b/ T_{thex} vs T_a, $y = -0.169x + 6.822$, $r^2 = 0.620$; T_{abex} vs T_a, $y = -0.098x + 3.206$, $r^2 = 0.516$
c/ (T_{abex}/T_{thex}) vs T_a

A paired t-test reveals that thoracic temperature is larger than abdominal temperature (n=17, T=8.67, p<0.001) during flight.

A regression of the proportion of the abdominal temperature excess relative to the thoracic temperature excess (T_{abex}/T_{thex}) against ambient temperature shows that (T_{abex}/T_{thex}) is independent of ambient temperature (Figure 5.10c) (n=11, R^2=0.16, p=0.108). This suggests that abdominal temperature excess remains a constant proportion of the thoracic temperature excess as ambient temperature increases, i.e. that haemolymph shunting is not in operation.

b/ *E. pertinax*

E. pertinax would not fly at ambient temperatures below 10 °C (even when stimulated). *E. pertinax* took off and flew at a range of ambient temperatures of 11 to 29 °C. Figure 5.11a & b shows plots of thoracic and abdominal temperatures, and of thoracic and abdominal temperature excesses against ambient temperature. No difference was observed between the sexes, and mass and thoracic width had no significant influence on thoracic temperature (covariance analysis not shown here). Again, both thoracic and abdominal temperatures are positively correlated with ambient temperature (regressions: T_{th}, n=11 R^2=0.99, p<0.001; T_{ab}, n=11, R^2=0.99, p<0.001). The gradient of T_{thex} against ambient temperature is different from zero, but not that of T_{abex} (regressions: T_{thex}, n=11, R^2=0.42, p<0.032; T_{ab}, n=11, R^2=0.019, p=0.68). *E. pertinax* flying in the laboratory do not maintain a constant thoracic temperature over this range of ambient temperatures. However, thoracic temperature does not parallel ambient temperature, although abdominal temperature does. *E. pertinax* achieves thoracic temperatures of around 13 °C at ambient temperatures of 11 °C. This temperature excess of about 2 °C, generated by endothermy alone, is reduced to about 0.8 °C at 29 °C. Thus, *E. pertinax* is able to thermoregulate slightly in the laboratory.

A paired t-test reveals that thoracic temperature is larger than abdominal temperature (n=11, T=4.25, p=0.0017).

A regression of (T_{abex}/T_{thex}) against ambient temperature shows that (T_{abex}/T_{thex}) is independent of ambient temperature (Figure 5.11c) (n=11, R^2=0.24, p=0.123). This suggests that haemolymph shunting is not in operation.

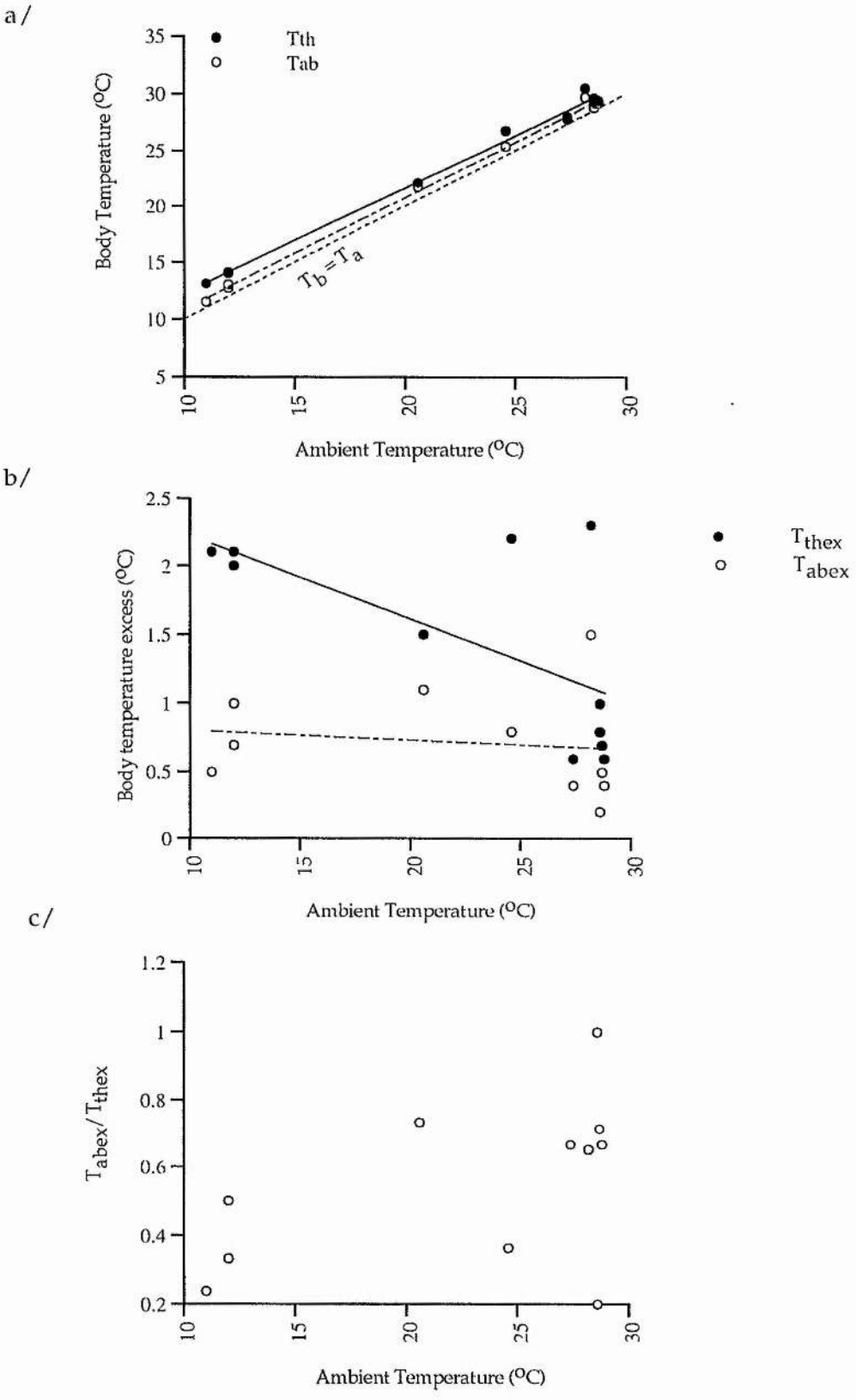


Fig. 5.11 *E. pertinax* "grab and stab" in the laboratory
a/ T_{th} vs T_a, $y = 0.939x + 2.831$, $r^2 = 0.994$; T_{ab} vs T_a, $y = 0.993x + 0.876$, $r^2 = 0.998$
b/ T_{thex} vs T_a, $y = -0.061x + 2.831$, $r^2 = 0.416$; T_{abex} vs T_a, $y = -0.007x + 0.876$, $r^2 = 0.019$
c/ T_{abex}/T_{thex} vs T_a

c/ Comparison of the two species

A covariance analysis of the effect of ambient temperature, species and the interaction between ambient temperature and species on thoracic temperature was carried out (Table 5.13). It shows that there is a species effect: *E. tenax* achieve higher temperature excesses than *E. pertinax* in flight in the laboratory. There is also an interaction between species and ambient temperature, which suggests that for the two flies, the regression line of thoracic temperature versus ambient temperature has a different gradient: thermoregulatory abilities in flight of these two species differ; *E. tenax* is a better thermoregulator than *E. pertinax* (this is clearly seen on Figures 5.10a and 5.11a; the gradient of the regression line of thoracic temperature on ambient temperature is less close to unity in *E. tenax* than in *E. pertinax*).

Table 5.13 "Grab and stab" in the laboratory: covariance analysis on thoracic temperature for ambient temperature, species and the interaction between T_a and species

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	1361.63	1218.80	1218.80	1192.92	<0.001
Species	1	17.47	11.96	11.96	1.70	0.002
T_a*Species	1	4.57	4.57	4.57	4.47	0.045
Error	24	24.52	24.52	1.02		
Total	27	1408.19				

Fitted means for T_{th}

	Mean (°C)	Stdev (°C)
E. tenax	23.47	0.25
E. pertinax	21.65	0.33

B/ Stable flight temperature

Both mass and thoracic width are likely to have some influence on SFT, and both should have a positive relationship with SFT. Heavier flies have a higher wing loading and need more muscle work to fly; larger flies have a smaller surface area to volume ratio and are less affected by convective cooling. Thus, both mass and thoracic width were used in this analysis. Season is not a significant factor and was omitted from the analysis.

SFT values were calculated with both equilibrium and ambient temperatures as baselines to investigate if taking one rather than the other would change any claim of thermoregulation in flight. The results of the present experiments are not shown graphically as they are similar to those of the "grab and stab" experiment.

Ambient temperature ranged from 11.0 to 34.7 °C and equilibrium temperature from 11.0 to 34.0 °C for *E. tenax*. For *E. pertinax*, ambient temperature ranged from 10.7 to 34.0 °C and equilibrium temperature from 10.7 to 33.0 °C.

a/ *E. tenax*

A covariance analysis of the effect of equilibrium temperature, thoracic width, mass, sex, feeding state, the interactions between sex and thoracic width and between sex and mass on SFT was carried out (Table 5.14). Again, a significant relationship between SFT_{ex} and equilibrium temperature would be an indication of thermoregulation in flight, thus the same covariance analysis was done for stable flight temperature excess (SFT_{ex}). Only the results for equilibrium temperature change, so the other factors are not shown in Table 5.15.

SFT has a positive relationship with equilibrium temperature: *E. tenax* do not fly at a constant thoracic temperature over this range of ambient temperatures. SFT_{ex} has a negative relationship with equilibrium temperature: the difference between SFT and temperature is larger at low temperature than at high. Therefore, *E. tenax* thermoregulate in flight to some extent. Both SFT and SFT_{ex} have a positive relationship with thoracic width once the other factors have been controlled for: large flies maintain higher temperature excesses than small ones in flight.

Table 5.14 Covariance analysis on SFT for T_{eq}, Th_w, mass, sex, feeding state, the interactions between sex and Th_w and between sex and mass in *E. tenax* (T_{eq} as the baseline)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	1131.13	903.69	903.69	493.78	<0.001
Th_w	1	0.44	8.56	8.56	4.68	0.042
Mass	1	0.18	3.83	3.83	2.10	0.163
Sex	1	1.05	5.45	5.45	2.98	0.099
Feeding	1	2.95	5.03	5.03	2.75	0.112
Sex*Th_w	1	3.53	5.99	5.99	3.27	0.085
Sex*mass	1	2.47	2.47	2.47	1.35	0.258
Error	21	38.43	38.43	1.83		
Total	28	1180.19				

Table 5.15 Covariance analysis on SFT_{ex} for T_{eq}, Th_w, mass, sex, feeding state, the interactions between sex and Th_w and between sex and mass in *E. tenax* (T_{eq} as the baseline)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	24.70	19.72	19.72	10.78	0.004

The same covariance analysis was carried out with ambient temperature instead of equilibrium temperature for SFT (Table 5.16) and for SFT_{ex} (Table 5.17). The results are very similar to those with equilibrium temperature as the baseline. SFT increases with temperature whereas SFT_{ex} decreases: the gradient of the regression line of SFT on ambient temperature is lower than 1. Using ambient temperature instead of equilibrium temperature as the external temperature the flies are exposed to similarly allows the detection of thermoregulatory abilities in flight in *E. tenax*. Both SFT and SFT_{ex} increase with thoracic width.

Table 5.16 Covariance analysis on SFT for T_a, Th_w, mass, sex, feeding state, the interactions between sex and Th_w and between sex and mass in *E. tenax* (T_a as the baseline)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	1134.76	906.95	906.95	541.49	<0.001
Th_w	1	0.61	9.03	9.03	5.39	0.030
Mass	1	0.01	4.35	4.35	2.60	0.122
Sex	1	0.57	5.81	5.81	3.47	0.077
Feeding	1	2.33	4.48	4.48	2.67	0.117
Sex*Th_w	1	3.16	6.55	6.55	3.91	0.061
Sex*mass	1	3.58	3.58	3.58	2.14	0.159
Error	21	35.17	35.17	1.67		
Total	28	1180.19				

Table 5.17 Covariance analysis on SFT_{ex} for T_a, Th_w, mass, sex, feeding state, the interactions between sex and Th_w and between sex and mass in *E. tenax* (T_a as the baseline)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	40.42	31.28	31.28	18.68	<0.001

Regressions of SFT against equilibrium temperature and ambient temperature were obtained and compared:

$$SFT = 5.33 + 0.869\ T_{eq}\ (n=31, R^2=0.96, T_{eq}\ p<0.001)$$

$$SFT = 5.72 + 0.839\ T_a\ (n=31, R^2=0.96, T_a\ p<0.001)$$

There is no difference between the gradients of the two regression lines (paired t-test, n=62, T=0.64, p>0.5). Thus, using either temperature for reference does not change the relationship between SFT and temperature.

These data were compared with the data obtained from the "grab and stab" experiment in the laboratory (T_a as the baseline) to check the accuracy of the "grab and stab" method (Table 5.18). The results show that the experimental procedure used to determine thoracic temperature in flight does not seem to influence the relationship between thoracic

temperature and ambient temperature in *E. tenax*, as neither the experimental procedure factor or the interaction between ambient temperature and experimental procedure are significant predictors of SFT.

Table 5.18 SFT and "grab and stab" compared for *E. tenax* with T_a as the baseline. Covariance analysis on thoracic temperature for T_a , experiment and the interaction between experiment and T_a

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	2024.79	1946.69	1946.69	1245.34	<0.001
Experiment	1	9.37	1.86	1.86	1.19	0.282
T_a*exp.	1	0.05	0.05	0.05	0.03	0.866
Error	44	68.78	68.78	1.56		
Total	47	2102.99				

b/ *E. pertinax*

The same covariance analyses using equilibrium temperature as for *E. tenax* were carried out (Tables 5.19 and 5.20).

When the other factors are controlled for, SFT is positively correlated with equilibrium temperature, and SFT_{ex} has a negative relationship with equilibrium temperature. Thus, *E. pertinax* do not fly at a constant thoracic temperature over this range of temperatures, but the thoracic temperature excess in flight is higher at low than at high temperature. *E. pertinax* thermoregulate in flight to some extent, but this ability is probably limited as the gradient of the regression line of SFT_{ex} on temperature is not highly significantly different from zero ($p = 0.038$). Both SFT and SFT_{ex} are positively correlated with thoracic width and have an almost significant positive relationship with mass. Sex is also a significant predictor of SFT: females have higher SFTs than males. Therefore, large flies maintain a higher thoracic temperature in flight than small flies, and females fly with higher thoracic temperatures than males.

Table 5.19 Covariance analysis on SFT for T_{eq}, Th_w, mass, sex, feeding state, the interactions between sex and Th_w and between sex and mass in *E. pertinax*(T_{eq} as the baseline)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	762.03	458.36	458.36	383.09	<0.001
Th_w	1	1.51	13.87	13.87	11.60	0.011
Mass	1	3.18	5.93	5.93	4.95	0.061
Sex	1	14.49	13.70	13.70	11.45	0.012
Feeding	1	0.06	0.06	0.06	0.05	0.835
Error	7	8.38	8.38	1.20		
Total	12	789.64				

Fitted means for T_{th}

	Mean (°C)	Stdev (°C)
Male	21.11	1.01
Female	25.32	0.49

Table 5.20 Covariance analysis on SFT_{ex} for T_{eq}, Th_w, mass, sex, feeding state, the interactions between sex and Th_w and between sex and mass in *E. pertinax* (T_{eq} as the baseline)

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_{eq}	1	1.702	7.836	7.836	6.55	0.038

SFT was also measured with ambient temperature as the baseline for *E. pertinax*. For clarity, the results are not shown here but are similar to those for *E. tenax*: there was no difference between the gradients of the regression lines of the plots of SFT on ambient temperature and on equilibrium temperature; the experimental procedure ("grab and stab" or "free" flight) did not influence the relationship between SFT and ambient temperature.

The species were not compared as the purpose of these experiments was to investigate any influence of the experimental procedures.

C/ "Grab and stab" in the field

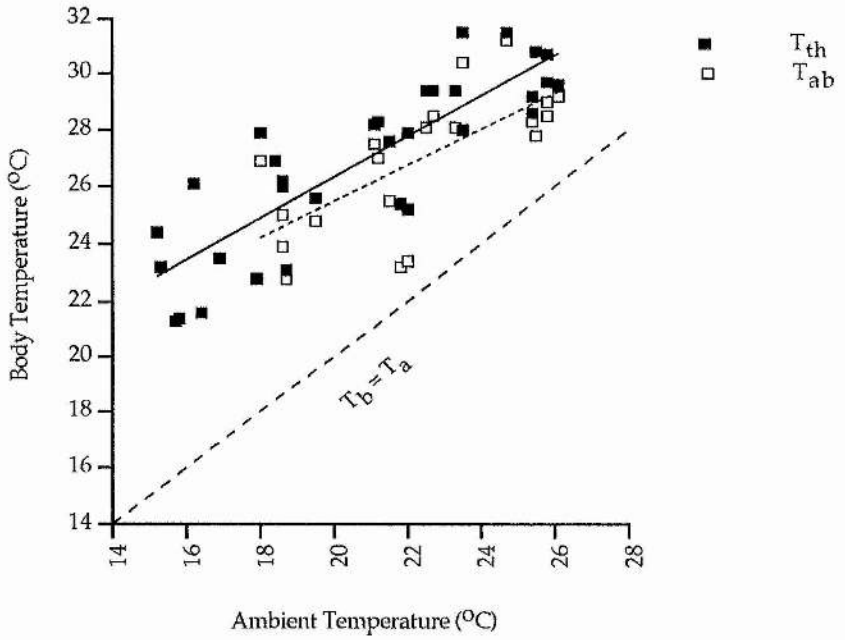
a/ *E. tenax* in flight

Figure 5.12a & b shows plots of thoracic and abdominal temperatures and of thoracic and abdominal temperature excesses on ambient temperature for flying *E. tenax*. Both thoracic temperature and abdominal temperatures are positively correlated with ambient temperature (T_{th}: n=32, R^2=0.72, p<0.001; T_{ab}: n=20, R^2=0.49, p<0.001) and thoracic and abdominal temperature excess have a negative relationship with ambient temperature (T_{thex}: n=32, R^2=0.28, p<0.002; T_{abex}: n=20, R^2=0.24, p=0.028). Thus, *E. tenax* do not maintain a constant thoracic temperature in flight in the field at ambient temperatures ranging from 15.2 to 26.1 °C, but their thoracic temperature excess is greater at low temperature than at high: the gradients of the regression lines of thoracic and abdominal temperatures over ambient temperature are lower than 1. Therefore, *E. tenax* exert some control over their thoracic and abdominal temperatures while flying.

Thoracic temperature is higher than abdominal temperature (paired t-test, n=20, T=8.01, p<0.0001). The proportion of abdominal temperature excess relative to thoracic temperature excess is not correlated with ambient temperature (regression, n=20, R^2=0.05, p=0.321).

A covariance analysis (Table 5.21) was carried out to investigate the influence on the logarithm of thoracic temperature of the logarithm of mass, ambient temperature, sex and the interactions between sex and ambient temperature and between sex and the logarithm of mass (thoracic width was not determined). The logarithm of mass does not have an effect on the logarithm of thoracic temperature, but there is a significant interaction between sex and the logarithm of mass: the relationship between the logarithm of thoracic temperature and the logarithm of mass is different in males and females.

a/



b/

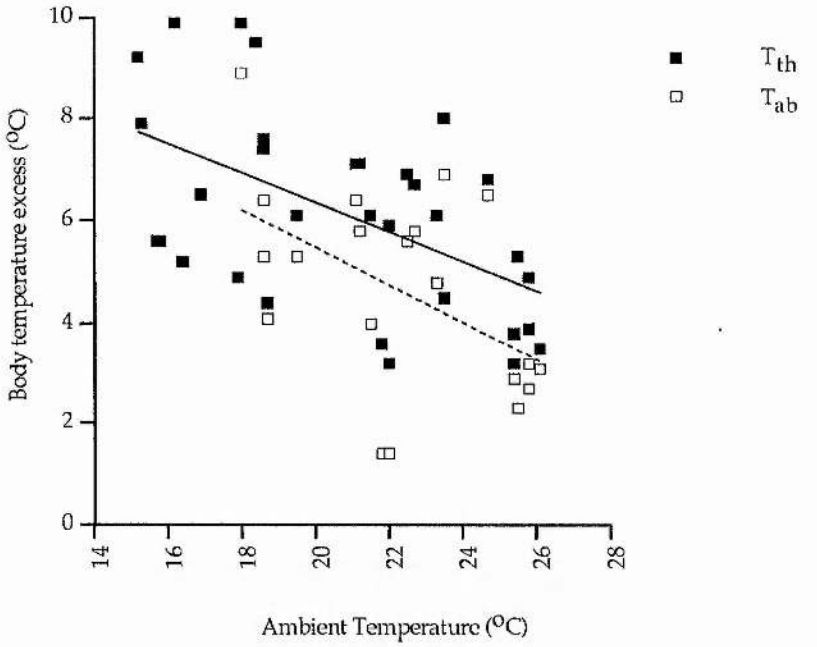


Fig 5.12 *E. tenax* "grab and stab"in flight in the field

a/ T_{th} vs T_a, $y = 0.719x + 11.941$, $r^2 = 0.724$;
T_{ab} vs T_a, $y = 0.636x + 12.761$, $r^2 = 0.491$
b/ T_{thex} vs T_a, $y = -0.286x + 12.074$, $r^2 = 0.282$;
T_{abex} vs T_a, $y = -0.364x + 12.761$, $r^2 = 0.240$

Table 5.21 "Grab and stab" in flying *E. tenax* in the field. Covariance analysis on $logT_{th}$ for T_a, sex, the interactions between sex and T_a and between sex and logmass.

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	0.056	0.053	0.053	74.79	<0.001
Logmass	1	0.00003	0.0008	0.0008	1.13	0.298
Sex	1	0.0005	0.0034	0.0034	4.80	0.038
Sex*T_a	1	0.0036	0.0036	0.0036	5.08	0.033
Sex*logmass	1	0.000001	0.000001	0.000001	0.00	0.967
Error	26	0.018	0.018	0.0007		
Total	31	0.078				

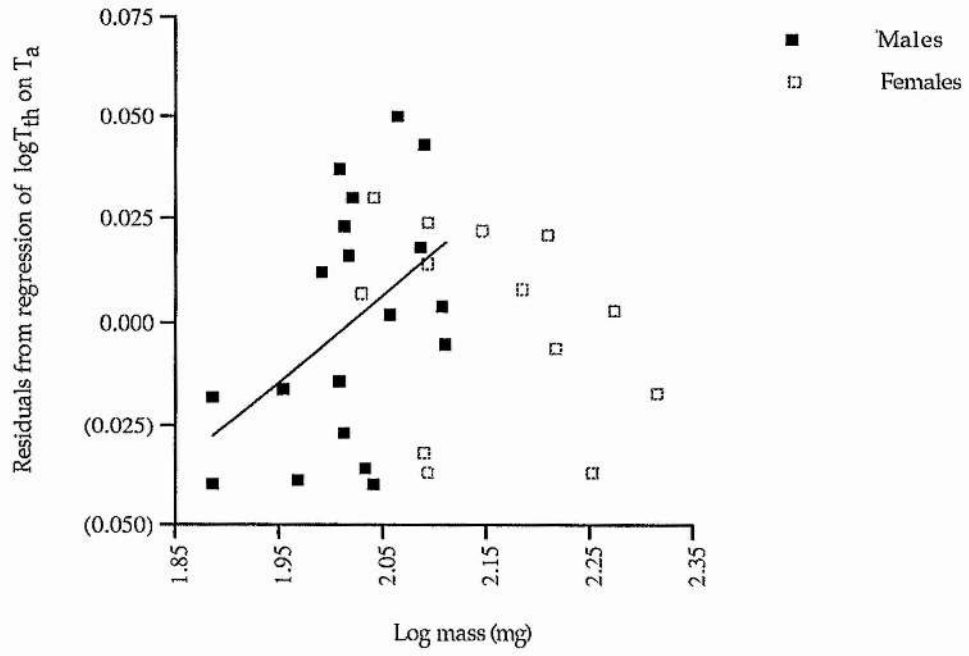
In view of these results, the sexes were separated. The multiple regression of the logarithm of thoracic temperature on ambient temperature and the logarithm of mass for females shows a positive relationship between the logarithm of thoracic temperature and ambient temperature, but no relationship between the logarithm of thoracic temperature and the logarithm of mass (n=13, R^2=0.76, T_a p<0.001, logmass p=0.353): mass does not influence thoracic temperature in flight in female *E. tenax*. However, for males, a positive relationship between the logarithm of mass and the logarithm of thoracic temperature was found (n=19, R^2=0.77, T_a p<0.001, logmass p=0.051): heavy males fly with a higher thoracic temperature than light ones. Figure 5.13a shows the relationship between the logarithm of mass and the residuals of the regression of the logarithm of thoracic temperature on ambient temperature for the two sexes.

Three flies were caught and their thoracic temperature measured when they were leaving the overwintering site in early spring. The three points are shown on Figure 5.14 where "grab and stab" results for laboratory and field experiments are compared.

b/ *E. tenax* "grab and stab" laboratory and field data compared

Figure 5.14 shows the relationship of thoracic and abdominal temperature with ambient temperature for flying *E. tenax* in the laboratory and in the field (summer and winter). The results of the covariance

a/



b/

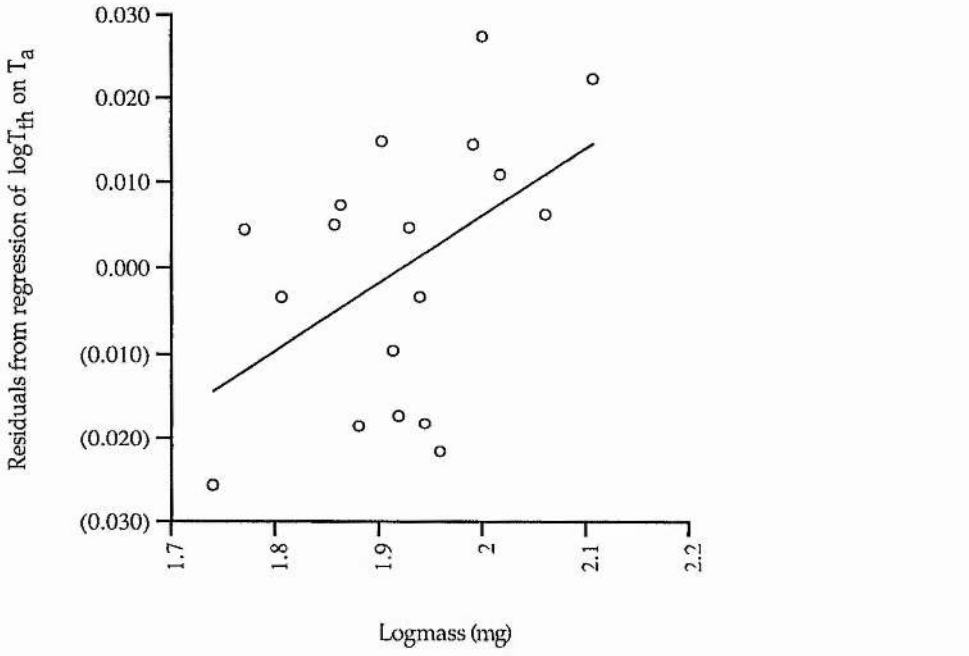
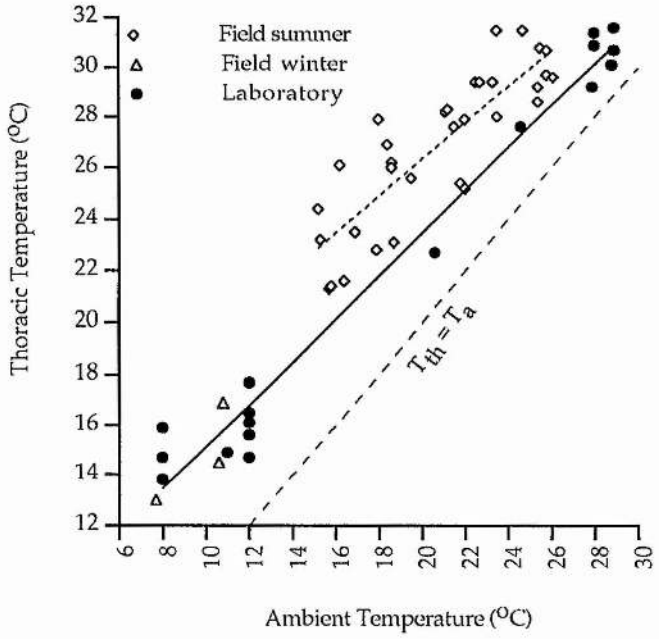


Fig. 5.13 "Grab and stab"flying: influence of mass on thoracic temperature

a/*E. tenax* Residuals of $logT_{th}$ on T_a versus logmass:
males, $y = 0.209x - 0.423$, $r^2 = 0.207$; females, not significant

b/ *E. pertinax* Residuals of $logT_{th}$ on T_a versus logmass (both sexes),
$y = 0.0.79x - 0.152$, $r^2 = 0.231$

a/



b/

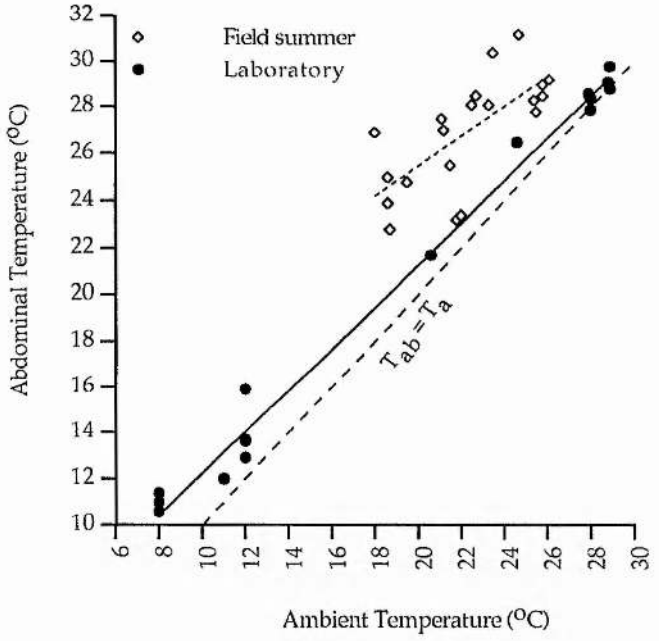


Fig. 5.14 *E. tenax* "grab and stab" in flight

a/ T_{th} versus T_a: field summer, $y = 0.719x + 11.941$, $r^2 = 0.724$; laboratory, $y = 0.831x + 6.822$, $r^2 = 0.975$
b/ T_{ab} versus T_a: field summer $y = 0.636x + 12.761$, $r^2 = 0.491$; laboratory, $y = 0.902x + 3.206$ $r^2 = 0.989$

analysis of the effect of ambient temperature and experimental condition (excluding winter data) are shown in Table 5.22. From Table 5.22 and Figure 5.14, it is clear that higher thoracic temperatures are achieved in the field. Results for abdominal temperature are similar and are not shown here. However, thermoregulatory ability is not influenced by the experimental procedure (the interaction between ambient temperature and experimental procedure is not significant).

Table 5.22 "Grab and stab" in flying *E. tenax*, comparison of field (summer) and laboratory data. Covariance on thoracic temperature for T_a, experiment and the interaction between T_a and experimental procedure.

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	1220.48	716.92	716.92	317.36	<0.001
Experiment	1	86.73	18.05	18.05	7.99	0.007
T_a*Exp.	1	3.74	3.74	3.74	1.66	0.205
Error	44	99.40	99.40	2.26		
Total	47	1410.35				

c/ *E. tenax* feeding in the field

Flies were caught while feeding on flowers. Thoracic temperature has a positive relationship with ambient temperature, and thoracic temperature excess has a negative relationship with ambient temperature:

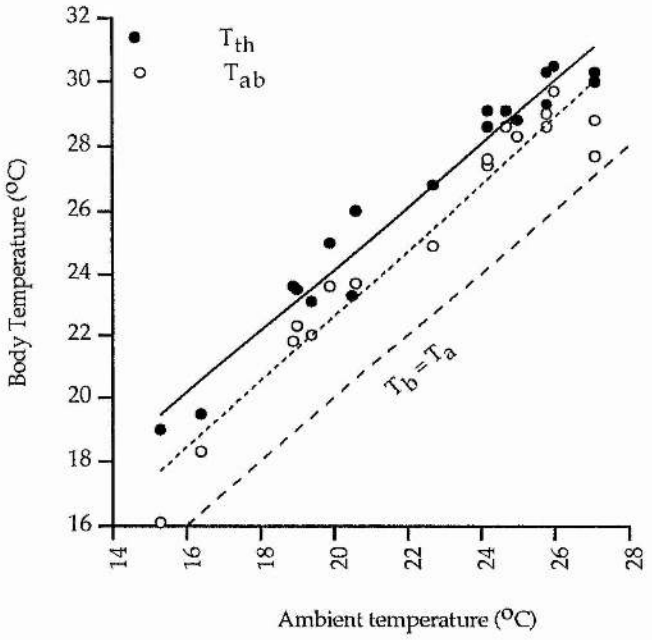
$$T_{th} = 0.675\ Ta + 12.6 \quad (n=24, R^2=0.79, p<0.001)$$

$$T_{thex} = -0.331\ T_a + 12.6 \quad (n=24, R^2=0.46, p<0.001)$$

Thus, while feeding *E. tenax* do not maintain a constant thoracic temperature over a range of ambient temperatures of 15.2 - 26.8 °C, but the thoracic temperature excess maintained is higher at low ambient than at high ambient temperature (thoracic temperature does not parallel ambient temperature), implying some thermoregulation.

d/ *E. pertinax* in flight

Figure 5.15a & b shows plots of thoracic and abdominal temperatures and of thoracic and abdominal temperature excesses on ambient temperature for flying *E. pertinax*. Both thoracic temperature and abdominal temperature are positively correlated with ambient temperature (T_{th}: n=18, R^2=0.96, p<0.001; T_{ab}: n=17, R^2=0.99, p<0.001).



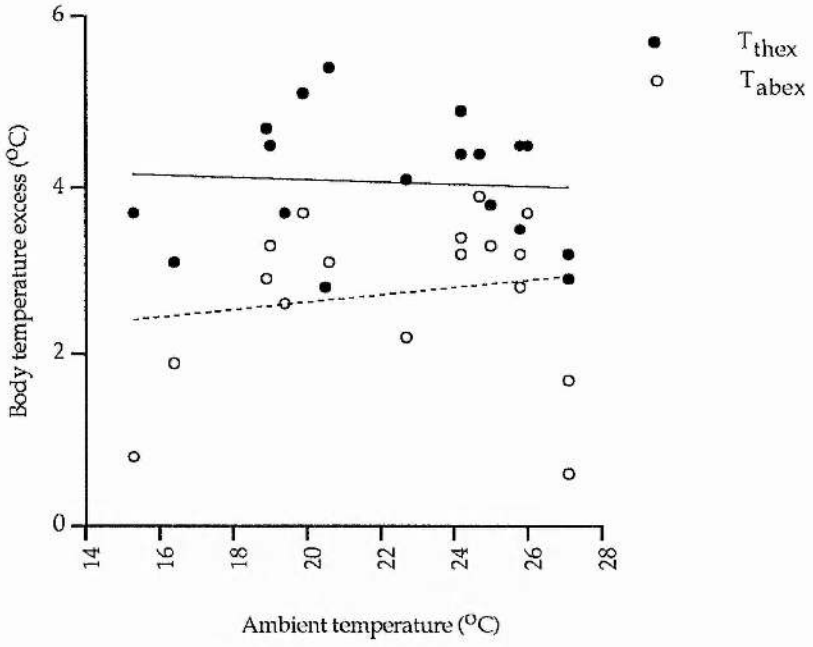


Fig 5.15 *E. pertinax* "grab and stab"in flight in the field

a/ T_{th} vs T_a, $y = 0.987x + 4.360$, $r^2 = 0.957$;
T_{ab} vs T_a, $y = 1.044x + 1.736$, $r^2 = 0.944$
b/ T_{thex} vs T_a, $y = -0.013x + 4.360$, $r^2 = 0.004$;
T_{abex} vs T_a, $y = 0.044x + 1.736$, $r^2 = 0.029$

Thoracic temperature and abdominal temperature excesses are not negatively correlated with ambient temperature (T_{thex}: n=18, R^2=0.004, p=0.804; T_{abex}: n=17, R^2=0.029, p=0.515). Therefore, both thoracic and abdominal temperatures are parallel to the isothermal line. Thus, *E. pertinax* flying in the field do not thermoregulate in flight over a range of temperature of 15.0 - 27.1 °C.

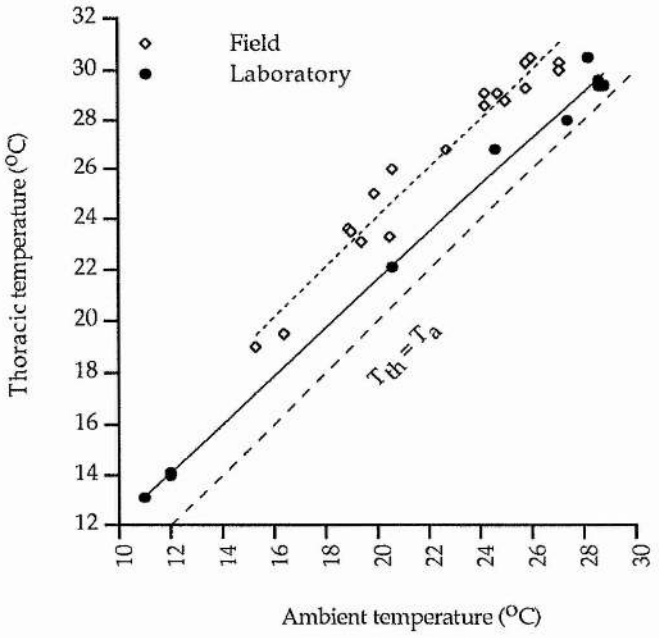
Thoracic temperature is higher than abdominal temperature (paired t-test, n=17, T=8.72, p<0.0001). The proportion of abdominal temperature excess relative to thoracic temperature excess is not correlated with ambient temperature (n=17, R^2=0.06 , p=0.335), i.e. haemolymph shunting could not be demonstrated.

Sex was found to have no influence on the logarithm of thoracic temperature (covariance analysis not shown here), but there were only four females in the sample. A multiple regression of the logarithm of thoracic temperature on ambient temperature and the logarithm of mass shows a positive relationship between both these factors and ambient temperature (regression, n=18, R^2=0.96, T_a p<0.001, logmass p=0.051). Figure 5.13b shows the relationship between the logarithm of mass and the residuals of the regression of the logarithm of thoracic temperature on ambient temperature. Thus, big *E. pertinax* fly with a higher thoracic temperature than small ones, but males and females fly with similar thoracic temperatures.

e/ *E. pertinax* "grab and stab" laboratory and field data compared

Figure 5.16a & b shows the relationship of thoracic and abdominal temperature with ambient temperature for flying *E. pertinax* in the laboratory and in the field. The results of the covariance analysis of the effect of ambient temperature and experimental condition are shown in Table 5.23 (interaction between ambient temperature and experimental condition omitted as not significant). From Table 5.23 and Figure 5.16, it is clear that higher thoracic temperatures are achieved in the field than in the laboratory and that the experimental technique has no effect on the relationship between thoracic temperature and ambient temperature. Results of the covariance analysis for abdominal temperature are similar and are not shown here.

a/



b/

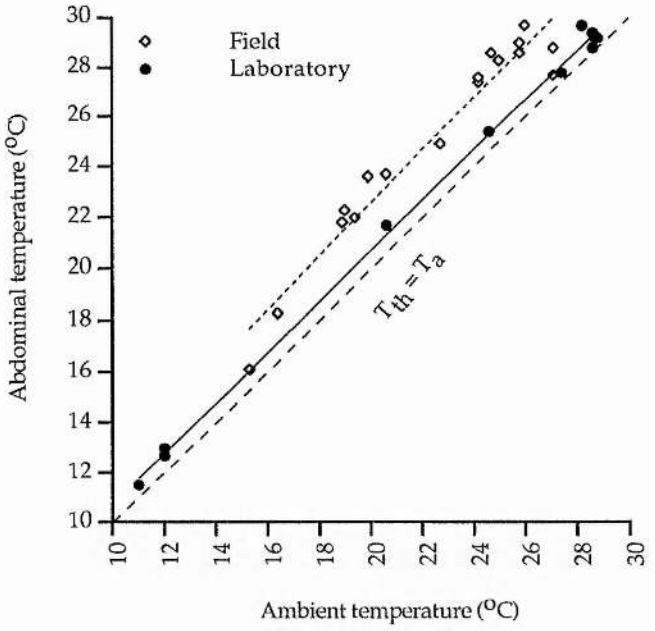


Fig. 5.16 *E. pertinax* "grab and stab" in flight

a/ T_{th} versus T_a: field , $y = 0.987x + 4.360$, $r^2 = 0.957$; laboratory, $y = 0.939x + 2.831$, $r^2 = 0.994$
b/ T_{ab} versus T_a: field $y = 1.044x + 1.736$, $r^2 = 0.944$; laboratory, $y = 0.993x + 0.876$, $r^2 = 0.998$

Table 5.23 "Grab and stab" in flying *E. pertinax* comparison of field and laboratory data. Covariance analysis on thoracic temperature for T_a and experiment

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	714.00	609.84	609.84	1176.24	<0.001
Experiment	1	46.16	46.16	46.16	90.00	<0.001
Error	26	13.34	13.34	0.51		
Total	28	773.50				

f/ *E. pertinax* feeding in the field

Thoracic temperature has a positive relationship with ambient temperature, and thoracic temperature excess has no relationship with ambient temperature:

$$T_{th} = 0.861\ T_a + 6.45\ (n=10, R^2=0.90, p<0.001)$$

$$T_{thex} = -0.139\ T_a + 6.45\ (n=10, R^2=0.20, p=0.197)$$

Therefore, while feeding, *E. pertinax* have thoracic temperatures that parallel ambient temperature.

g/ *E. pertinax* hovering (field)

Figure 5.17a is a graph of thoracic temperature and abdominal temperature against ambient temperature (ambient temperature: 12.0 - 26.4 °C). Both thoracic and abdominal temperatures have a positive relationship with ambient temperature:

$$T_{th} = 0.32\ T_a + 23.8\ (n=50, R^2=0.16, p=0.004)$$

$$T_{ab} = 0.90\ T_a + 6.25\ (n=26, R^2=0.54, p<0.001)$$

Thoracic temperature excess has a negative relationship with ambient temperature, whereas abdominal temperature excess has no relationship with ambient temperature:

$$T_{thex} = -0.68\ T_a + 23.8\ (n=50, R^2=0.46, p<0.001)$$

$$T_{abex} = -0.10\ T_a + 6.25\ (n=26, R^2=0.01), p=0.553)$$

These results show that although thoracic temperature is not maintained constant in hovering flight in *E. pertinax* over this range of ambient temperatures, it does not parallel ambient temperature: thoracic temperature excess is higher at low than at high ambient temperature (gradient of the regression line of thoracic temperature on ambient

temperature lower than 1). However, abdominal temperature parallels ambient temperature. On Figure 5.17a, thoracic temperature data points for ambient temperature below 16 °C are lower than expected when looking at the rest of the data. Figure 5.17b shows the same data with these points separated. In this case, thoracic temperature has no relationship with ambient temperature, and thoracic temperature excess has a negative relationship with ambient temperature:

$$T_{th} = -0.001\ T_a + 30.5\ (n=46, R^2=0, p=0.992)$$

$$T_{thex} = -1.00\ T_a + 30.5\ (n=46, R^2=0.60, p<0.001)$$

Thus, at ambient temperatures above 16 °C, *E. pertinax* maintains a constant thoracic temperature around 30.5 °C. Below 16 °C, thoracic temperature increases with ambient temperature. The lowest ambient temperature at which *E. pertinax* was seen to hover is 12 °C. Between 12 and 16 °C, hovering flights are only of short duration and depend on the sun being out.

No relationship between thoracic temperature and mass was found, but it might be significant that the flies hovering at ambient temperature below 16 °C were amongst the heaviest caught.

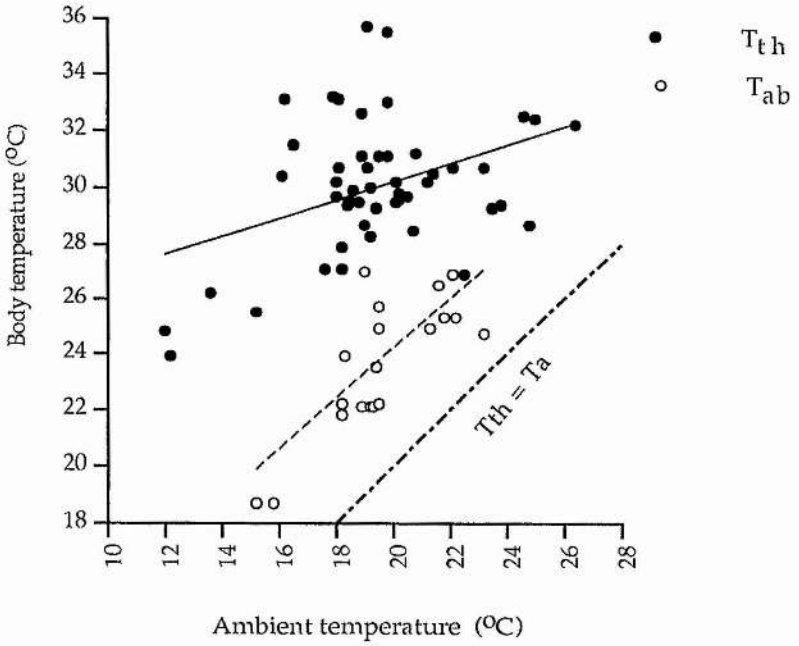
h/ "Grab and stab" in flight - the two species compared (field data)

A covariance analysis (Table 5.24) reveals that there is a species effect on thoracic temperature; looking at Figure 5.18a & b, it is clear that *E tenax* maintain higher thoracic and abdominal temperature excesses than *E. pertinax*. There is also an interaction between species and ambient temperature which reflects the different thermoregulatory abilities of the two species.

Table 5.24 "Grab and stab" T_{th} in flight, comparison of the two species (field data). Covariance analysis on T_{th} for T_a, species and the interaction between T_a and species

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	388.06	424.80	424.80	222.42	<0.001
Species	1	33.76	17.20	17.20	9.00	0.004
T_a*Species	1	10.46	10.46	10.46	5.47	0.024
Error	46	87.86	87.86	1.91		
Total	49	520.13				

a/



b/

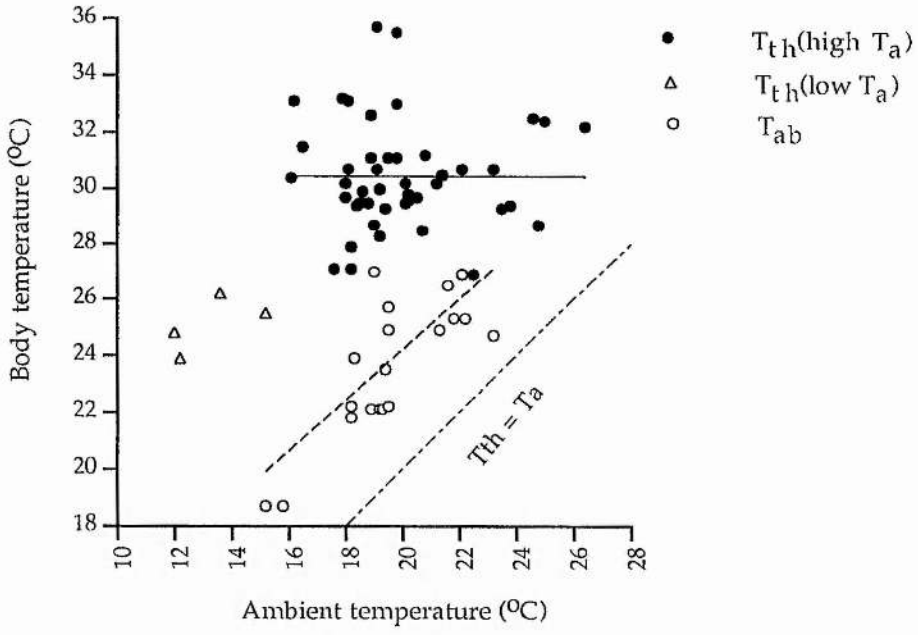
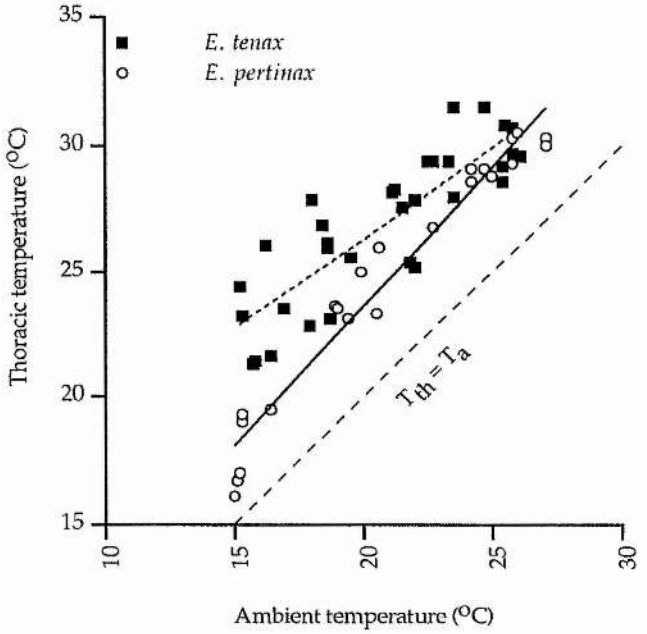


Fig. 5.17 *E. pertinax* "grab and stab" hovering flight

a/ T_{th} vs T_a (all data), $y = 0.318x + 23.833$, $r^2 = 0.157$;
T_{ab} vs T_a, $y = 0.898x + 6.250$, $r^2 = 0.542$
b/T_{th} vs T_a (T_a>16 °C), $y = -0.001x + 30.484$, $r^2 = 0$; T_{ab} vs T_a(as above)

a/



b/

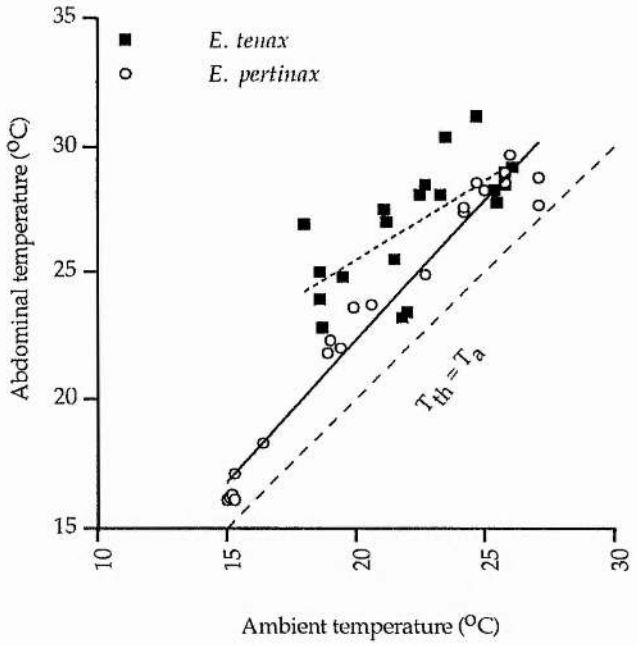


Fig. 5.18 "Grab and stab" in flight *E. tenax* and *E. pertinax* compared

a/ T_{th} vs T_a: *E. tenax*, $y = 0.719x + 11.941$, $r^2 = 0.724$;
E. pertinax, $y = 1.108x + 1.445$, $r^2 = 0.956$
b/T_{ab} vs T_a: *E. tenax*, $y = 0.636x + 12.761$, $r^2 = 0.491$;
E. pertinax, $y = 1.111x + 0.112$ $r^2 = 0.966$

The results for abdominal temperature are similar (Table 5.25)

Table 5.25 "Grab and stab" T_{ab} in flight comparison of the two species (field data). Covariance analysis on T_{ab} for T_a, species and the interaction between T_a and species

Source	DF	SeqSS	AdjSS	ADjMS	F	P
T_a	1	284.50	241.88	241.88	110.17	<0.001
Species	1	33.11	20.42	20.42	9.30	0.004
T_a*Species.	1	14.26	14.26	14.26	6.49	0.016
Error	33	2.45	2.45	2.20		
Total	36	404.31				

D/ Summary

With these two eristalines, the thoracic temperatures measured by the "grab and stab" method accurately represent thoracic temperatures in flight.

Both sexes of *E. tenax* and *E. pertinax* can fly with varying thoracic temperatures. However, the regression lines of thoracic and abdominal temperatures on ambient temperature do not follow the isothermal line in *E. tenax*, but they do in *E. pertinax*: *E. tenax* thermoregulate to some extent in flight, but *E. pertinax* do not.

The same is true for feeding flies in the field.

However, male *E. pertinax* hovering maintain a constant thoracic temperature of 30.5 °C over ambient temperatures ranging from 16 to around 27 °C. Abdominal temperature is not controlled in hovering *E. pertinax*.

E. tenax maintain higher temperature excesses than *E. pertinax*. Large *E. pertinax* and male *E. tenax* maintain higher temperature excesses than small ones. No relationship between size and thoracic temperature was found for female *E. tenax*.

5.3.4 Haemolymph shunting in *E. tenax*

In view of the results obtained in the "grab and stab" experiment, only *E. tenax* seems to be a good thermoregulator in flight and would be expected to use haemolymph shunting (even though this could not be

directly demonstrated with the results from the "grab and stab" experiment). *E. pertinax* could be using haemolymph shunting during hovering flight, but this was not investigated because of the experimental difficulties involved.

A/ Results from simultaneous measurement of thoracic and abdominal temperatures

Thoracic and abdominal temperatures in tethered flight were recorded by thermocouples implanted in the thorax and the abdomen of the flies (Figure 5.1). Several flies were tested at low (12-14 °C), medium (18- 20 °C) and high (30-35 °C) ambient temperature. But, due to the difficulty of inducing tethered flight in these flies, only five usable recordings were obtained. From the recordings, models of the changes of thoracic and abdominal temperatures (at high and low ambient temperatures only) before, during, and after flight bouts (tethered flight) could be constructed (Figures 5.19 & 5.20). These are explained below. Figures 5.21 & 5.22 show real thoracic temperature and abdominal temperature traces at high and low ambient temperature for two flies.

a/ Changes in thoracic and abdominal temperatures at high ambient temperature

E. tenax being quite a "leaky" fly (Chapters 3 and 4), body temperature at rest is usually a few degrees (up to about 2.5°C) below ambient at high ambient temperature because of evaporative cooling. For this model, equilibrium temperature is taken as the baseline.

At high ambient temperature, flies do not need to warm up before flight. When the fly starts flying, thoracic temperature increases until the fly reaches its stable flight temperature. As the fly is subject to forced convective cooling during flight (here no forward movement, so forced convective cooling results only from the wings beating), the increase in thoracic temperature means that heat production is greater than convective cooling. At STF, both are in equilibrium. Meanwhile, abdominal temperature follows a similar pattern, only the rise is smaller and it is the result of either conduction from the thorax, haemolymph shunting, or both. It also reaches a stable temperature. Thoracic temperature increase from VFT is typically 4-5 °C at ambient temperature around 33 °C, whereas abdominal temperature only rises by a degree

Celsius at most. When the fly stops flying, the effect of forced convective cooling is lost: thoracic temperature increases by 1 or 2 °C very rapidly (a few seconds), as does abdominal temperature, but to a lesser extent. Abdominal temperature does not get much higher than ambient temperature. Moreover, abdominal temperature remains high while thoracic temperature falls, and it was observed in a few flies that abdominal temperature reaches its peak later than thoracic temperature (the maximum delay recorded was 84 s).

b/ Changes in thoracic and abdominal temperatures at low ambient temperature

At low ambient temperature, the fly has to warm up before it can take off. It has been demonstrated above that *E. tenax* uses endothermy (at least in some circumstances) to achieve the required thoracic temperature for flight. This is again clearly seen in the simultaneous thoracic temperature and abdominal temperature traces obtained at low ambient temperature. Before flight, thoracic temperature is increased by several degrees (shown as about 6 °C in the model, but sometimes more). Abdominal temperature does increase as well, but only by one or two degrees Celsius; this is probably the result of heat conduction from the thorax to the abdomen, but could also reflect haemolymph flow between thorax and abdomen which, although it might be kept to a minimum when it is important to keep heat in the thorax, is probably nevertheless essential for the transport of nutrients from the abdomen to the thorax. Just before flight, the difference between thoracic and abdominal temperature can be over 4°C. At the onset of flight, thoracic temperature drops very quickly to its STF under the effect of forced convective cooling (convective cooling is here greater than heat production). Abdominal temperature also decreases because of forced convective cooling. At the end of flight, the loss of forced convective cooling is reflected by a rapid increase of the thoracic temperature (not always back to its original level). Likewise, abdominal temperature rises. This increase in abdominal temperature, if only caused by the loss of convective cooling, should not reach a level higher than that at the start of the flight bout. However, it quite often does so, and this must reflect hot haemolymph transfer from the thorax. This is further demonstrated by the fact that, again, abdominal temperature often reaches its peak after thoracic temperature. Thereafter,

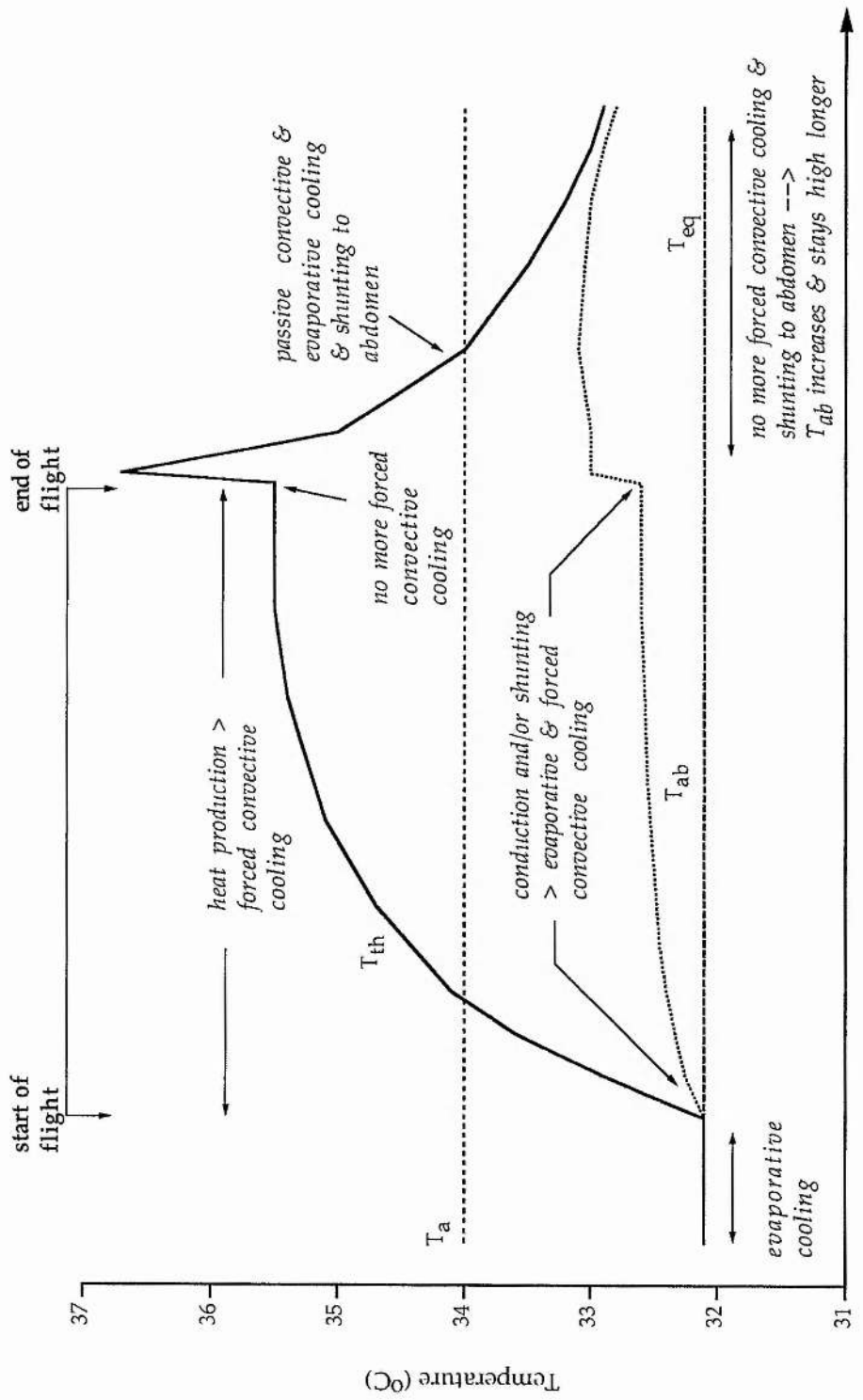


Fig. 5.19 Model of thoracic and abdominal temperature changes during a flight bout at high ambient temperature (34 °C) in females *E. tenax*

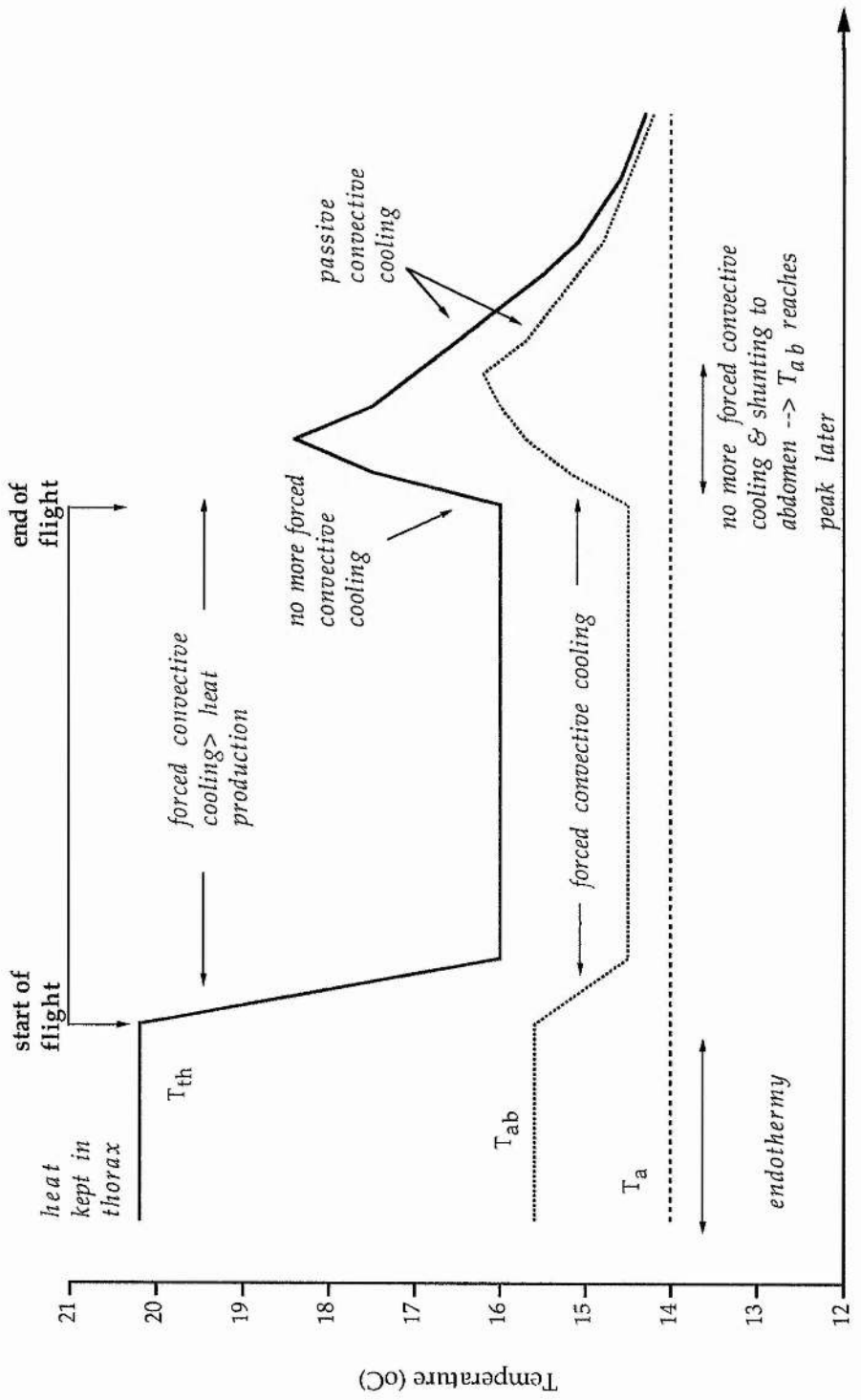


Fig. 5.20 Model of thoracic and abdominal temperature changes during a flight bout at low ambient temperature (14 °C) in females *E. tenax*

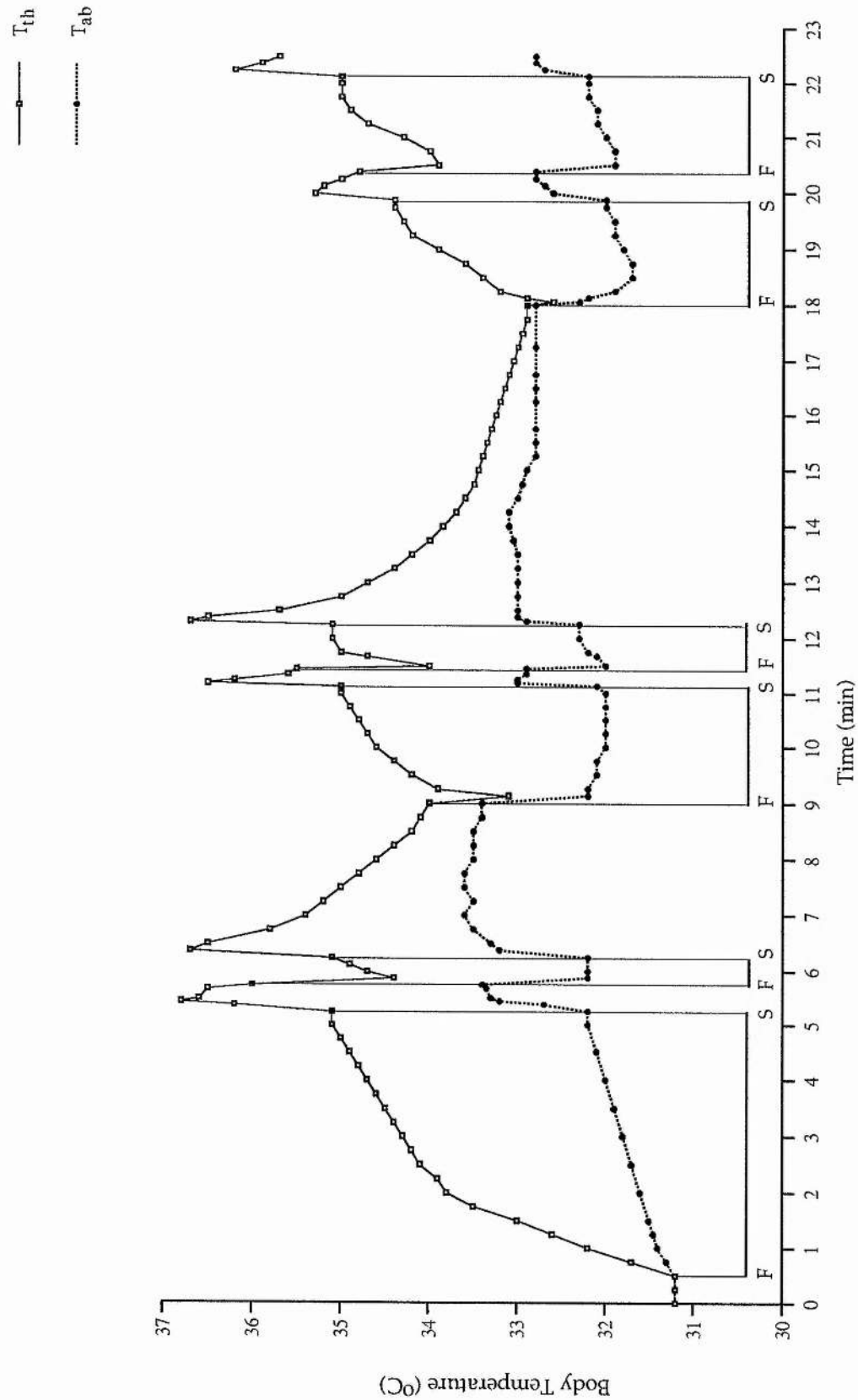


Fig. 5.21 Thoracic and abdominal temperatures over time for a female *E. tenax* at T_a = 34 °C. Solid bars indicate the duration of tethered flight (F = flight, S = stop).

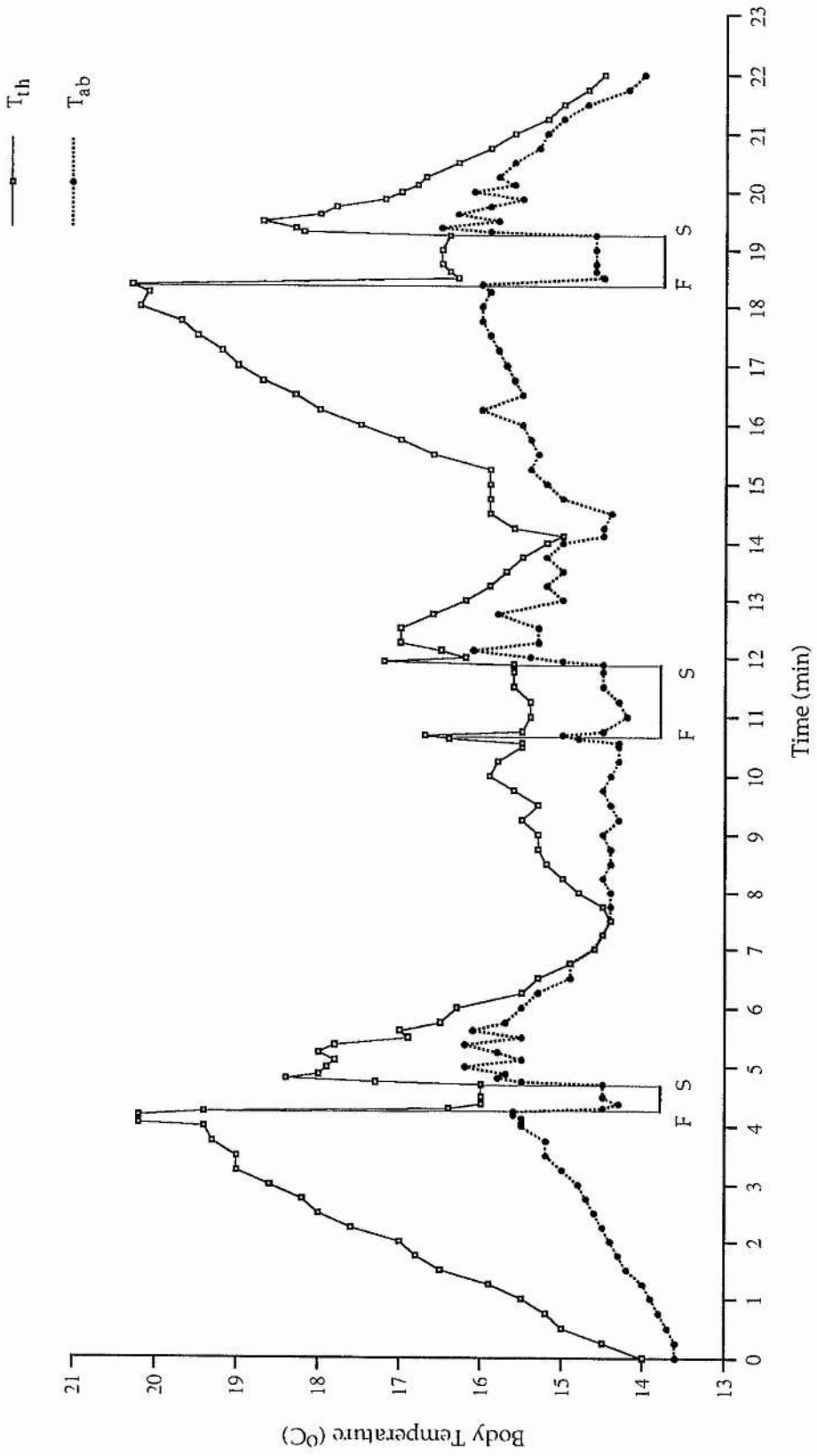


Fig. 5.22 Thoracic and abdominal temperatures over time for a female*E. tenax* at T_a = 14 °C. Solid bars indicate the duration of tethered flight (F = flight, S = stop).

both thoracic and abdominal temperatures decrease very rapidly after flight has ceased by passive convective cooling.

5.4 Discussion

5.4.1 Endothermy

When just looking at the mean endothermic warm-up rates, *E. tenax* and *E. pertinax* have similar rates. They also have similar rates to other insects of similar size (Table 5.26). However, when thoracic temperature, mass and thoracic width are controlled for, *E. tenax* have larger endothermic warm-up rates than *E. pertinax*. In other words, *E. tenax* of similar size and with the same thoracic temperature warm up faster than *E. pertinax* (Table 5.8). It can be suggested that because of their overwintering habits, *E. tenax* might need more efficient endothermic abilities than *E. pertinax*. *E. tenax* are often active early in the spring (and sometimes even in the winter) when temperatures are not sufficiently high to allow flight without warming up before hand (usually around 10-12 °C). As they come out of their overwintering site, they cannot rely on heat from the sun to warm up before flight: they can only fly if they can warm up endothermically. *E. pertinax* do not face the same constraints. The first adults are active in the late spring/early summer, but even if temperatures are cool, *E. pertinax* can rely on solar radiation to warm up prior to flying. Therefore, this difference in endothermic abilities might reflect ecological differences between the two species.

Table 5.26 Mean EWR of some insects of similar size to *E. tenax* and *E. pertinax*

Species	Mean body mass (mg)	T_a (°C)	Mean EWR and range (°C/min)	Source
Syrphids (bee- and wasp-mimics)	118	12.3-18.0	1.2-5.5	Morgan & Heinrich (1987)
E. tenax	**135.3**	**10.5-27.0**	**2.83 (0.78-9.30)**	**This thesis**
E. pertinax	**99.3**	**8.7-27.2**	**2.85 (0.71-7.29)**	**This thesis**
Chlorotabanus mexicanus	28 (thx)		3.9 (3.3-4.5)	May (1976a)
Tabanus lineola	32 (thx)		6.7 (6.5-6.9)	May (1976a)
Leucotabanus albibasis	35 (thx)		3.3	May (1976a)
Cryptotylus unicolor	58 (thx)		4.9	May (1976a)
Tachinid flies (*Nowickia* sp.)	130.4	15.0-18.0	2.59 (1.82-3.31)	Chappell & Morgan (1987)
Apis m. mellifera L.	89	22.0	4.8	Stone &Willmer (1989a)
Euglossa imperialis (Cockerell)	160	22.0	7.0	Stone &Willmer (1989a)
Anthophora plumipes (Pallas)	185	22.0	12.3	Stone &Willmer (1989a)
Amegilla sapiens (Cockerell)	117	22	3.75	Stone &Willmer (1989a)
Andrena nigroaenea (Kirby)	108	22.0	6.95	Stone &Willmer (1989a)
Colletes cunicularius (L.)	112	22	7.35	Stone &Willmer (1989a)
Megachile willoughbiella (Kirby)	121	22.0	6.3	Stone &Willmer (1989a)
Bombus lapidarius (L.)	136	22.0	6.1	Stone &Willmer (1989a)

The curvilinear shape of the endothermic warm-up traces (Figure 5.3) suggests that as thoracic temperature (and thus thoracic temperature excess) increases, the flies are subject to an increasing convective cooling effect which is not compensated for. There are two possibilities: the rate of endothermic warm-up remains constant with temperature or it increases but not sufficiently to counteract the increasing convective cooling effect. The present results show that endothermic warm-up rates have a positive relationship with thoracic temperature, and hence with ambient temperature. Warmer flies warm up faster than cooler ones. These rates represent the mean of several measurements from several warm-up bouts for single flies, where each measurement is taken at thoracic temperature equals equilibrium temperature. However, the changes in endothermic warm-up rate along single warm-up traces were also investigated for a few flies. In this case, the measurements were corrected for passive cooling for thoracic temperatures above equilibrium temperature. The results are not shown here, but they confirm that the endothermic warm-up rate increase with thoracic temperature during single endothermic bouts as well (and this eliminates the effect of ambient temperature), but not sufficiently to counteract the increasing convective cooling effect. It is advantageous for an insect to warm up as rapidly as possible in order to counteract the loss of heat through convective cooling and to limit the time during which it could not escape if attacked. However, it seems that these two eristaline flies do not warm up at their maximum rate at all ambient temperatures, but that their endothermic warm-up rates depend on their thoracic temperature. This dependence of the endothermic warm-up rates on thoracic temperature probably reflects a physiological constraint of muscle contraction (warm-up is achieved by shivering): muscles contract at a higher rate when they are warmer, as was shown by Heinrich and Bartholomew (1971) in sphinx moths.

As mentioned in Chapter 1, the relationship between endothermic warm-up rate and size in insects has been somewhat controversial. This relationship depends on the changes with size of thermogenic abilities of the tissues and of heat losses. In the present study, only two species are investigated. When considering each species separately, the thermogenic ability of the tissues is constant. What is going to vary is heat losses as a result of changing surface area to volume ratios in relation to size. It has already been shown (Chapter 4) that heat losses decrease with size in

these two eristaline flies. Thus, it is expected that endothermic warm-up rates are positively correlated with body size in each species. For *E. tenax*, mass is not a significant predictor of endothermic warm-up rate (once thoracic temperature has been controlled for), but thoracic width is. As already mentioned, mass can vary greatly within individuals and does not necessarily accurately reflect body size (Chapter 2). Moreover, the relationship between mass and thoracic width differs between sexes in *E. tenax*. Thoracic width is a good measure of body size for the present purpose as we are interested in the muscles' ability to produce heat in the thorax and in the heat lost from the thorax. Thus, the positive relationship between endothermic warm-up rate and thoracic width in *E. tenax* reflects the decrease in heat loss with increasing size. For *E. pertinax*, the relationship between endothermic warm-up rate and size (both mass and thoracic width) seems to be going in the other direction. This is surprising as it has previously been shown (Chapter 4) that heat losses are negatively correlated with size in this species as well. This would suggest that larger flies, even though they have lower heat loss, cannot produce endothermic warm-up rates as high as small flies when the effect of thoracic temperature has been corrected for. A more likely explanation is that the small size of the sample is at the origin of this result. It is difficult to analyse the effect of several factors for a small sample as not enough subjects fall in the various categories. It is thus very likely that this result came up by chance. For example, the only big fly tested at high ambient temperature had a lower than average endothermic warm-up rate. When both species are grouped together, the assumption that muscle thermogenic ability is constant might not be true. If it varies, it becomes more difficult to predict the direction of the relationship between endothermic warm-up rate and size (hence, the differing results of the various studies). Here, the absence of a relationship when both species are analysed together does not necessarily imply that there is such a variation in muscles' thermogenic activity. It rather comes from the fact that the relationship between endothermic warm-up rate and thoracic width has a different direction in the separate species (probably due to the small sample size in *E. pertinax*). Thus, the positive relationship which was demonstrated by Morgan and Heinrich (1987) in some hoverflies could not be confirmed here.

The main effect of feeding seems to be the loss of the relationship between endothermic warm-up rate and size (significant interaction between feeding state and thoracic width in *E. tenax*; the absence of significance is probably due to the small sample size in *E. pertinax*). In addition, there is a decrease in endothermic warm-up rates after feeding in *E. tenax*, but not in *E. pertinax* . Various suggestions can account for the loss of the relationship between endothermic warm-up rate and thoracic width: the quantity of sugar water ingested by the flies was not controlled, and it is possible that the warming effort and the effect of evaporative cooling, for example, were altered to differing degrees amongst the flies as the result of feeding. In addition, fed flies contain more water. The evaporation of this water, passive and/or active, might explain the lower EWR after feeding in *E. tenax*.

Feeding also seems to influence the duration and the pattern of the endothermic warm-ups. Figure 5.2 shows the four main patterns of endothermy observed in *E. tenax* and *E. pertinax*. Often, a mixture of these was observed during a single trial. Flight attempts are not shown on this figure but were observed with all four patterns. The significance of these patterns is not known. It does not seem very likely that they would be observed in nature as endothermic warm-ups probably culminate in flight: they are more likely to result from the experimental procedure which restrains the flies. In the "free-flight" experiments, where the flies could take off, these patterns were not observed. Table 5.1 shows a sample of *E. tenax* endothermic warm-ups before and after feeding (similar results were obtained for *E. pertinax*). Unfed flies which did not warm-up were omitted. This shows that repetitive warm-ups are less common and are of shorter duration in unfed flies. Endothermic activity sometimes lasted for considerable periods (more than two hours - experiments had to be interrupted). Feeding did not always result in endothermy, as probably some flies were damaged when the thermocouple was inserted, but it seems to have an effect on the type of patterns, their repetition and duration.

5.4.2 Voluntary Flight Temperature

No effect of mass could be detected either in the separate species or with the two species together over the whole range of equilibrium temperatures, but a positive relationship between voluntary flight

temperature and mass was found for *E. tenax* when the range of equilibrium temperatures was restricted to a maximum of 15 °C (Fig. 5.7) (the small sample size for *E. pertinax* precluded such an analysis). A positive relationship between voluntary flight temperature and mass would be expected, as wing loading usually increases with mass (although this was not tested in this case). Also, larger individuals, due to smaller heat losses, can reach higher temperature excesses, particularly at low ambient temperature, when endothermic warm-up is necessary before take off. At higher ambient temperature, the thorax is hot enough to allow take-off: voluntary flight temperature is not necessarily related to mass. The difference between the species seems to stem from the fact that at low ambient temperature *E. tenax* has higher voluntary flight temperatures than *E. pertinax* (Fig. 5.9). The difference seems to disappear at higher ambient temperatures. This probably relates to the fact that the smaller *E. pertinax* do not need as high voluntary flight temperatures to take off at low ambient temperatures as *E. tenax*.

Experimental procedure did not influence voluntary flight temperature in *E. tenax*, but it did in *E. pertinax*. It is not quite clear why this is so. It seems that at low ambient temperature, voluntary flight temperature is higher in fixed than in "free" flight (Fig. 5.8) perhaps because the weight of the Styrofoam sphere led *E. pertinax* to increase its voluntary flight temperature. The mass of the sphere (around 30 mg) is relatively larger for *E. pertinax* than for *E. tenax*, and the latter might not have been so much influenced (when the data for the two flight types for *E. tenax* are looked at separately, the same trend, although not significant, is observed)(Fig. 5.6). Thus, it is possible that using fixed (or tethered) flight to determine voluntary flight temperature can lead to overestimates, especially in small insects.

Voluntary flight temperature is not maintained constant over the range of ambient temperatures at which *E. tenax* and *E. pertinax* normally fly. Voluntary flight temperature was found to vary from about 14.5 °C (the lowest VFT recorded at T_{eq} = 12.5 °C) to around 31°C (at T_{eq} = 34 °C) in *E. tenax*, and from about 13 °C (at T_{eq}=12.2 °C) to about 31 °C (at T_{eq}=33 °C) in *E. pertinax*. This represents a range of over 20°C. At low ambient temperature, these two eristalines (especially *E. tenax*) therefore have to warm up before they can take off. As ambient temperature increases, the need to warm up diminishes. Both flies can initiate flight over a wide

range of thoracic temperatures, but it was noted that at low ambient temperature (and at low thoracic temperature) the flies are slower and less agile than at high ambient temperature. This would suggest a possible thermal constraint preventing them from achieving high voluntary flight temperatures at low ambient temperatures. Also, in this experiment, the flies were cooled down before being released, and most of them took off at a thoracic temperature lower than the equilibrium temperature would be at such a high ambient temperature. This would not happen in the wild as the flies are likely to have a body temperature close to ambient temperature. Nevertheless, the relationships of voluntary flight temperature and voluntary flight temperature excess with equilibrium temperature should still be present. In this experiment, the flies could only rely on endothermic warm-up whereas they would be able to get some heat from the sun in the field. Therefore, it is likely that these eristalines are able to reach higher voluntary flight temperatures at low ambient temperatures in the field. However, overwintering *E. tenax* leaving overwintering sites can only rely on endothermic warm-up. Thus, the relationship between voluntary flight temperature and temperature at ambient temperatures below 12-14°C (ambient temperatures encountered in early spring) described here is very likely to be similar to that in the field.

5.4.3 Stable flight temperature and "grab and stab" in the laboratory

When these syrphids warm-up endothermically before flight, stable flight temperature is lower than voluntary flight temperature, reflecting the convective cooling effect associated with flight. At high ambient temperature, the flies initiate flight below stable flight temperature; thoracic temperature increases until it reaches stable flight temperature (Figures 5.21 and 5.22).

The results from the stable flight temperature experiment are very much in line with those from the "grab and stab" (in the laboratory) experiment. When a fly stops flying, there is a rapid increase in thoracic temperature because it is not subject to convective cooling any more. But thoracic temperature decreases quickly thereafter (see Figures 5.21 and 5.22, thoracic temperature traces during flight). The accuracy of the estimation of thoracic temperature in flight by "grab and stab" depends on

where in this phase thoracic temperature is measured. Here, no difference between the two experimental methods was found, suggesting that the delay associated with handling in the "grab and stab" experiment compensates for the rise of thoracic temperature at the end of flight. Also, it seems that the flies caught in the net did not warm-up endothermically, as was the case for the bees Stone and Willmer (1989b) investigated. If this had been the case, the "grab and stab" experiment would have shown better thermoregulation because thoracic temperature would have increased more at low ambient temperature.

Using ambient temperature rather than equilibrium temperature (when it cannot be determined, as in the field) does not have any significant effect on the conclusions about the thermoregulatory abilities of these flies. As the difference between ambient and equilibrium temperature increases with ambient temperature, it was suspected that using ambient temperature rather than equilibrium temperature as the baseline might lead to the claim of better abilities than really exist (by showing a stronger negative relationship between stable flight temperature excess and temperature), but this was not the case.

5.4.4 Thermoregulatory abilities

A/ *E. tenax* and *E. pertinax* in flight

These results suggest that *E. tenax* is capable of some thermoregulation in flight. *E. pertinax*, on the other hand, does not seem to thermoregulate in flight in the field and thermoregulates only slightly in the laboratory.

However, thoracic temperature is not maintained constant in flight (in the laboratory experiment it ranges from 14 to 31.5 °C in *E. tenax* and from 13 to 30 °C in *E. pertinax*). As expected, the thorax, where heat is produced by the flight muscles, is always hotter than the abdomen in flight. *E. tenax* also maintain higher thoracic temperature excesses than *E. pertinax*. It seems that *E. tenax* (and *E. pertinax* to a much smaller extent) uses endothermy to warm up at the lower ambient temperatures to achieve thoracic temperatures high enough to support flight. Does it also actively regulates its thoracic temperature at high ambient temperature to avoid overheating? It is possible that water evaporation (passive and perhaps active) has an effect in reducing thoracic temperature at high ambient temperatures. Church (1964) suggested that water evaporation in

the desert locust *Schistocerca gregaria* could not account for thoracic temperature reduction in flight. However, the results obtained in this study, with much "leakier" insects (see Chapter 4), suggest that water evaporation could account for thoracic temperature depression. To prove such an effect would require further experiments with precise measurements of water loss and thoracic temperature in flight at various ambient temperature and humidities.

Another means to avoid overheating is by "shunting" haemolymph from the warm thorax to the cooler abdomen. Some heat must pass to the abdomen as abdominal temperature does not follow the isothermal line in *E. tenax*, but this process might not be actively controlled. Haemolymph shunting could not be directly demonstrated with the data obtained from the "grab and stab" experiment (Fig. 5.10c and 5.11c). As explained in the introduction, this does not necessarily mean that the flies do not use haemolymph shunting when they risk overheating. Very possibly the ambient temperatures to which they were exposed were not high enough to make them thermally stressed. Thus, further investigations, such as the haemolymph shunting experiment which will discussed below, are required to distinguish between these two possibilities. These were investigated in the haemolymph shunting experiment which will be discussed below.

B/ *E. tenax* and *E. pertinax* feeding in the field

The "grab and stab" results for feeding flies are very similar to those for flight: *E. tenax* thermoregulates while feeding, whereas *E. pertinax* does not seem to do so.

C/ *E. pertinax* hovering

E. pertinax does not thermoregulate in flight; but it seems to be a good thermoregulator, and an excellent thermoregulator at ambient temperatures above 16 °C, when hovering. Above 16 °C, *E. pertinax* is able to maintain a constant mean thoracic temperature of 30.5 °C (thus a maximum thoracic temperature excess of 14.5 °C). Below that threshold, thermoregulation, although present (maximum T_{thex} = 12.8 °C), is not as efficient. At low temperature, these flies hover only if the sun is out, suggesting that they rely (at least partly) on solar radiation as a source of heat to warm up before taking off. And they only hover for a short

duration; they might not be able to maintain the high thoracic temperature that seems necessary for hovering flight at these low ambient temperatures. When they land, they bask for a few minutes before taking off again. Above 16 °C, although the number of flies hovering depends on the presence of the sun, some individuals hover even with the sun in. Hovering flight can last several minutes (see Chapter 7).

Therefore, *E. pertinax* is able to thermoregulate while hovering, but does not do so in forward flight. During hovering flight, an insect is not subject to convective cooling resulting from forward flight, but is still exposed to considerable air flow because a mass of air equal to the body mass has to be accelerated downwards by the wings (Weis-Fogh 1975, Casey 1975). Thus, even if convective cooling is less than in forward flight, it will still have an effect on the thermal balance of hovering *E. pertinax*. Also, the lift gained in forward flight is absent, and more muscle power is needed to keep the insect airborne, hence more heat is produced (Pennycuick 1969). The combined effects would help to maintain an elevated thoracic temperature, but do not explain the maintenance of thoracic temperature at a constant level. Hovering flight was interspersed with short bouts of forward flight, sometimes to pursue a passing insect, sometimes with no clear reason. It is possible that these are used to cool the thorax down. For example, Heinrich and Buchmann (1986) showed that carpenter bees increase heat losses by flying faster. It would be interesting to observe if the frequency of such bouts of forward flight increases with ambient temperature. Also, abdominal temperature follows the isothermal line. Therefore, it seems that somehow the flow of heat from the thorax to the abdomen is prevented. Unfortunately, no data for abdominal temperature is available at ambient temperatures above 23.2 °C, so no information on the possible use of the abdomen as a heat sink is available. The variation, or non-variation, of wing beat frequency with ambient temperature should also be investigated as Harrison et al (1996) and Roberts et al (1998) have shown that honey bees and the bee *Centris pallida* reduce their wing beat frequency at high ambient temperature to decrease heat production.

The maintenance of a constant thoracic temperature during hovering flight has also been recognised in horse flies *Tabanus nigrivittatus* and *T. conterminus* (Schutz and Gaugler 1992) and in the tabanid *Hybomitra arpadi* (Smith et al 1994). *T. nigrivittatus* maintain their thoracic temperature at

28.3 ± 4.6 °C (early morning hovering males) and 36.7 ± 0.78 °C (late morning hovering males). *T. conterminus* hover with a thoracic temperature of 35.2 ± 0.28 °C, and *H. arpadi* with a thoracic temperature of 40 °C. It is possible that the success of a male *E. pertinax* depends so much in its ability to hover, and probably to defend a territory at the same time, that thermoregulation is crucial during that type of flight to ensure maximum ability (length of flight, agility, etc.) over an ambient temperature range as wide as possible.

D/ "Grab and stab" in flight - comparison of laboratory and field

The gradients of the regression lines of thoracic temperature on ambient temperature are the same for both experiments. However, it is clear that the thoracic temperature excesses of flies in the field are higher than those in the laboratory. In the laboratory, the only heat source available is the heat produced by the fly itself. In the field, heat can come from external sources as well, particularly solar radiation. Therefore, it is clear that these syrphids benefit from external heat gain prior to and during flight.

The three data points obtained when female *E. tenax* were leaving their overwintering site in early spring fit neatly amongst the data obtained in the laboratory at similar ambient temperatures. This makes sense, as in these field conditions, the flies can only rely on endogenously produced heat, as in the laboratory (there is no solar input).

E/ Effect of size on thoracic temperature excess

The SFT experiment shows that large flies maintain higher temperature excesses than small ones; thoracic width is here the best predictor of size. For *E. pertinax*, females do maintain higher temperature excesses in flight than males, and this can also be linked to the fact that for the same thoracic width, females are heavier than males. Thoracic width was not determined in the "grab and stab" experiment. Nevertheless, once ambient temperature has been controlled for, the logarithm of thoracic temperature has a positive relationship with the logarithm of mass for *E. pertinax*: heavy flies maintain higher thoracic temperature excesses than light ones. For *E. tenax*, the same relationship is true only for males. Figure 5.13a shows that heavy females, in particular, have low thoracic temperature excess. This probably reflects a higher mass for the same

thoracic width in some females (see Chapter 2). Thus, although heavy, these females are relatively small. These results show that mass is not necessarily the best size predictor, and that it might be better to include both thoracic width and mass when investigating the effect of size. Moreover, within a species, the two sexes might have a different relationship between size and the factor under investigation. Therefore, sex should be taken into account.

5.4.5 Haemolymph shunting

"Grab-and-stab" laboratory data do not suggest active haemolymph shunting in *E. tenax* at high ambient temperature as the regression lines from plots of the proportion of abdominal temperature excess relative to thoracic temperature excess is independent of ambient temperature. However, as was mentioned in the introduction, the absence of such a relationship does not mean that haemolymph shunting is not used. As *E. tenax* appears to thermoregulate to some degree in flight, a more direct investigation of this process was carried out.

Simultaneous recordings of thoracic and abdominal temperatures do not completely confirm an active haemolymph shunting. These recordings do show that heat is transferred to the abdomen, maybe during flight but certainly after flight, as abdominal temperature increases above starting levels and often reaches its peak after thoracic temperature. However, it cannot be concluded that this process is actively controlled. The cooling patterns described at high ambient temperature strongly suggest haemolymph shunting. The thorax cools down due to passive convective, and possibly evaporative cooling, and the transfer of heat to the abdomen. The abdomen can only rely on evaporation to cool down, as abdominal temperature is usually below ambient temperature (Fig. 5.19 and 5.21). Most probably, the heat lost that way can help in cooling the thorax, but to what extent this process is efficient is not known. The abdomen remains warm longer than expected, probably because hot blood keeps being transferred to it; abdominal temperature can only start falling when the effect of evaporative cooling is larger than that of heat transfer, or when haemolymph stops being shunted.

At low ambient temperature (Fig 5.10 and 5.22), either haemolymph flow is restricted during warm-up to reduce heat loss, and facilitated at the end of flight (controlled process), or activity causes an increase in

haemolymph flow (passive process). Abdominal temperature does not remain high after flight: either the shunting of haemolymph is not actively maintained to the contrary of what happens at high ambient temperature, or the heat transferred to the abdomen is not sufficient to prevent abdominal temperature falling due to passive convective cooling.

All these patterns of body temperature change can be explained by increased haemolymph flow with exercise, which is not necessarily a controlled process. It is possible that haemolymph shunting is actively increased at high temperature, but my results do not allow this conclusion to be drawn with certainty. Even the fact that abdominal temperature does not decrease as rapidly at high ambient temperature as at low ambient temperature could reflect a less effective cooling by evaporation alone, or again a passively increased blood circulation. Moreover, haemolymph shunting seems to occur at low ambient temperature, when there is no risk of overheating.

Haemolymph shunting was also indicated by the fact that flies that warm up endothermically (when only the thorax is heated) cool at the same rate as flies heated by a lamp (the whole body is heated) (see Chapter 4): both cool down from the whole body.

Haemolymph shunting has been demonstrated in the moth *Manduca sexta*, in cuculiine moths and in bumblebees (Heinrich 1970, 1971a, 1971b, 1976, 1987; Heinrich and Esch 1994). The counter current system which was described in Chapter 1 is by-passed when the insect risks over heating. Hot haemolymph is thus transferred in the abdomen where heat can be lost to the environment. Similar recordings as those presented here were obtained by Stavenga et al (1993) in tethered blowflies (*Calliphora vicina*) using thermal imaging. These authors recognise that the increase in abdominal temperature during and after flight is due to haemolymph circulation. Heinrich (1993) reports that in an unpublished study using thermovision pictures, he did not detect any haemolymph shunting to the abdomen in *E. tenax*. In contrast, in the present work, haemolymph shunting has been shown to be present. It probably helps cool the flies, but it might (as May, 1976b, suggested for dragonflies) just be the result of an uncontrolled process, i.e. blood flow rate increases with body temperature, rather than being a controlled process put in operation when there is a risk of overheating.

In this chapter, it has been demonstrated that both *E. tenax* and *E. pertinax* are capable of endothermic warm-up. *E. tenax* has higher endothermic warm-up rates than *E. pertinax*. Both regulate their voluntary flight temperature to some extent, but *E. pertinax* has lower voluntary flight temperatures than *E. tenax*. Only *E. tenax* thermoregulates moderately in flight and while feeding on flowers. However, *E. pertinax* appears to be an excellent thermoregulator in hovering flight, when defending a territory. Thermoregulation might partly be achieved by haemolymph shunting in *E. tenax*, but it seems unlikely that this process is actively controlled.

In general, larger flies need higher voluntary flight temperatures to take off (at low ambient temperature) and achieve higher temperature excesses in flight. Larger flies also generate higher endothermic warm-up rates. Again, the size factor can be either thoracic width or mass. It is sometimes worth investigating the effect of both size factors and also checking for sex differences.

The validity of the "grab and stab" method to estimate body temperatures in free ranging insects was confirmed for these flies.

Chapter 6 - Overwintering

6.1 Introduction

Eristalis tenax is one of the few insects that overwinter as adults in Scotland. Another hoverfly, *Eristalis aenus*, also exhibits this behaviour. Female *E. tenax* are inseminated and look for a suitable site in autumn. A few males also overwinter, but the others die. Males overwintering are scarce in Scotland: only one was found throughout this three year project. However, Ellis (1937) reports a sighting of both sexes overwintering. Females spend the winter in the chosen site and come out in the spring to lay their eggs.

Eggs need to develop and be fertilized before they are laid. Kendall and Stradling (1972) dissected some overwintering females and found that the ovaries and basal oocytes were small and undeveloped. Feeding the females with honey and pollen, and keeping them in a heated glasshouse, led to the development of these structures and eggs were laid between eight and fifteen days after overwintering was terminated. A minimum of five weeks is required before the new adult generation emerges from eggs, and the duration of development is temperature sensitive (Heal 1979a).

The flies that emerge in late spring/early summer are not very numerous. *E. tenax* really appear in large numbers in a second generation from August and persist until around mid-November, depending on the weather, when females again begin overwintering.

E. tenax has been sighted feeding in England in December (Hastings 1988, Gilbert pers. comm.), in Japan in December (Kato 1943) and resting on sunlit limestone and flying short distances during warm days in January 1957 at Wrzosy (Poland) (Siuda 1963). No reports of active drone flies during these months have been found for Scotland. These sightings could be of flies which have not yet started to overwinter, or have already

come out. In particular in December in more southern regions, if the weather has been mild, it would not be too surprising to encounter flies which have not yet begun to overwinter considering that *E. tenax* is quite often still active in November in Scotland. Later sightings would suggest early emerging.

This study follows on from Siuda's (1963) who made ecological observations of overwintering *E. tenax* in caves in the vicinity of Cracow (Poland), in a much harsher climate than in Scotland. The aims of the present investigation were to identify the climatic factors that influence the start and end of overwintering in Scotland. A description of the sites used by *E. tenax* for overwintering and of the microenvironment offered was obtained.

These flies could survive the winter either by selecting a suitable habitat or by adapting their physiology or by a mixture of both. Some flies were therefore collected for laboratory investigations of water balance and thermoregulation. In Chapter 3, we saw that overwintering females physiologically restrict their rate of water loss, but are nevertheless dehydrated. Do they also select a habitat that helps them to limit water losses? How do the flies cope with cold weather? The production of anti-freeze agents or the freezing tolerance (Davenport 1992, Zachariassen 1985) of the flies were not investigated here. However, as will be seen, the temperature of the overwintering sites remains well above freezing, so such specific physiological/biochemical adaptations would not be needed.

The number of flies in two parts of an overwintering site (Newark Castle, Fife) were monitored over a whole season and were examined in relation to climatic data. The records looked at individual crevices as well as total number of flies in these two sites. It was intended to investigate potential movement within and between crevices and immigration/emigration from these data.

This study was limited to one whole season because a suitable site, close enough to St Andrews (which would allow regular visits) was only discovered well into the second winter of this project. The data are used in conjunction with less comprehensive data obtained from the other two winters.

6.2 Materials and methods

6.2.1 Looking for overwintering sites

One site, Yellowcraig, was known by Graham Rotheray (National Museums of Scotland, Edinburgh) to shelter overwintering *E. tenax*. Some other sites were discovered by bat specialists who were recording hibernating bats. Others were found by investigating potential sites around St Andrews (Fife) and in East Lothian. This involved looking with a torch in cracks and crevices in rocks and between stones in caves, mines and ruins. Newark Castle (Fife) is a ruin on the Fife coast which shelters *E. tenax* during winter. This site was used for a more detailed study because it is a convenient distance from St Andrews, thus allowing frequent visits.

6.2.2 Climate recording

Air temperature (T_a) and relative humidity (RH) were measured with a hand held temperature humidity meter HMI 31 (Vaisala Ltd, UK) held about 1.5 m above the ground. Crevice temperature and relative humidity were also obtained from the Vaisala probe placed in the crevices. In some cases, relative humidity was measured with the "wiggly wire" technique, as described in Chapter 3. The wires were placed within the crevices. Light levels were obtained from a hand held light meter (LX-101, Lutron).

"Tiny talk" humidity and temperature data loggers (Gemini Data Loggers Ltd. UK) were used to record the climate continuously (every 30 minutes). This failed for humidity as the probes are not designed to work in humidities above 98.5 %. The very high humidity in the crevices, coupled with the low temperatures, probably resulted in some water condensation on the probe. One temperature probe was placed outside in the east wall (Figure 6.1a) and one in a crevice in Room 2 of Newark Castle (Figure 6.1b) The probes had to be hidden in wide crevices as the Castle is freely open to the public and was often frequented by children using it as a play area. For that reason, the probe outside was only protruding slightly from the wall and was somewhat sheltered: the temperatures recorded might be higher than actual air temperatures. They are nevertheless in accordance with temperatures recorded at Fife Ness

a/

b/

Fig. 6.1 Continuous measurement of the climate with data loggers at Newark Castle
a/ Outside: probe placed between stones in the east wall
b/ Probe inside a crevice in Room 2

(about 10 miles north along the coast from Newark Castle) by the Meteorological Office. Room 2 of the castle was chosen for the inside recording as it is the darkest, thus the more suited for hiding the probe.

The Meteorological Office in Edinburgh kindly provided temperature records for January-April 1997 and August 1997-May 1998 at Fife Ness. These are not average temperatures but rather day and night minima and maxima. Day maxima and night minima were used here and represent extreme daily temperatures.

6.2.3 Recording flies' abundance in Newark Castle

As many as possible of the crevices were investigated for overwintering *E. tenax*. Occupied crevices were recorded (with details of their shape, depth and position) on a plan of the room. At each visit, all the crevices which at any time contained some flies were looked at, and the number and position of the flies noted. A mark with a felt pen was made on the wall. This census method ensured a systematic recording of overwintering flies. Recording was done from September 1997 to April 1998, usually every two weeks except during December. Flies were found in 3 of the "rooms" of the castle which were more or less intact (still had a roof on). Rooms 1 and 2 were used throughout this period. The census of Room 3 was stopped in January as some flies had to be collected for laboratory experiments. In winter 1997, when the site was discovered, a similar but less accurate and less systematic recording was done in March and April.

6.2.4 Marking flies

Marking flies to follow their eventual movement within and between crevices was attempted with a few flies. A dot or dots of enamel paint (Humbrol Ltd, UK) of various colours were placed on the flies' dorsal thorax or abdomen using a grass stalk dipped in the paint.

6.2.5 Analysis and graphs

As this work covered only one full season and included sporadic records from two other seasons, a statistical analysis would be meaningless. Inference of the factors influencing overwintering *E. tenax*

was drawn from observations, records and graphs of climatic data and flies' abundance over time.

6.3 Results

6.3.1 Description of the overwintering sites

Typical overwintering sites are crevices in caves and spaces between stones in old buildings and ruins. These provide a shelter from adverse climatic conditions in the winter. During the course of this study, several sites have been identified in North East Fife and East Lothian (Table 6.1).

Table 6.1 Overwintering sites used by *E. tenax* in north East Fife and East Lothian

Name	Type of site	Position	Map Ref.
North East Fife			
Whallyden	Old mine	Inland	NO 363 043
Kilrenny Cave	Cave	Seaside	NO 601 058
Newark Castle	Ruin	Seaside	NO 518 011
East Lothian			
Yellowcraig	Cave	Seaside	NT 592 860
Crichton Castle	Ruin	Inland	NT 380 630
Tantallon Castle	Castle	Seaside	NT 595 850

Yellowcraig is well known by Graham Rotheray for being used regularly by *E. tenax* for overwintering. Whallyden, Crichton Castle and Tantallon Castle were discovered to shelter drone flies by people looking for hibernating bats; it is worth mentioning that except in Whallyden, Yellowcraig and Kilrenny, bats were found hibernating in the larger crevices of all the sites. The investigation of several potential sites led to the finding of overwintering *E. tenax* at Newark Castle and Kilrenny.

Finding overwintering drone flies is not an easy task, and many of potential sites were visited with no success. Moreover, the flies cannot usually be seen without looking in crevices with a torch. I will describe below the characteristics of the sites, and in particular of Yellowcraig and Newark Castle which were studied more extensively. Why drone flies were absent from many potential sites, and why some sites only sheltered a few flies while others had tens of them (size difference of the sites not being the important factor) is not known. However, it seems that *E. tenax* goes back to the same sites year after year. For example, Graham Rotheray (pers. comm.) has found overwintering *E. tenax* in Yellowcraig for many years. Also, every site which was found, early in this study, to shelter *E. tenax* during winter did so the next winter(s) as well.

The cave at Yellowcraig is about 14 m long, 7 m wide and 2.5 m high, and is within volcanic rock (Figure 2a & b). There is a single wide opening which allows some light in, but neither of the side walls receive much light (Table 6.2). Most of the crevices are found in the east wall and the roof of the cave, and this is where overwintering flies were seen; none was found on the west wall. The cave is very humid, and water can often be seen dripping from the walls and roof.

Newark Castle is a ruin, and only four lower "rooms" are more or less intact (Figure 6.3 a & b). Three of them were studied in more details (Figure 6.3c). Room 1 (about 4 x 5 x 2.5m) has four openings: 2 in the east wall and 2 in the west (Figure 6.3c, d & e). This room is draughty, light and quite humid. Room 2 (about 5 x 5 x 2.5 m) has only one opening in the west, which makes it a dark, well sheltered room (Figure 6.3e & f); it is very humid, and water could be seen dripping from the wall and roof on several occasions. Room 3 (around 5 x 5 x 2.5 m) represents an intermediate for climatic conditions between Rooms 1 and 2. With two openings in the west wall but none in the east, it is well protected from the wind, allows some light in and is again very humid (Figure 6.3f). The overwintering flies were well distributed both between and within the three rooms, although they tended to be more numerous near openings.

The records shown below (Table 6.2) for Yellowcraig and Newark Castle were taken on different days and are thus not comparable; they are only to be used for a general description of the type of habitat selected by the drone fly for overwintering.

a/

b/

Fig. 6.2 Yellowcraig: a cave used by overwintering *E. tenax*
a/ View of the entrance of the cave at Yellowcraig (East Lothian)
b/ View from the cave

a/

b/

Fig. 6.3 Newark Castle, Fife

c/

d/

Fig. 6.3 Newark Castle, Fife; View of the west side
c/ The entrance of Room 3 is seen just under the foot of the arch; the entrance of Room 2 is on its left. The two other openings on the left are from Room 1 (we can see straight through Room 1)
d/ The two openings in the west wall of Room 1 (and one in the east wall)

Table 6.2 Climatic records at Yellowcraig and Newark Castle on two different days

		Light	T_a	Air RH	Comments
		(W/m^2)	(° C)	(%)	
Yellowcraig					
Entrance		9.6	5.2	84.5	Bright,
East wall mid-way through		0.4	5.3	84.5	sunny day;
West wall mid-way through		0.1	5.4	84.6	28.1.96; at
Bottom of cave		0.1	5.4	84.5	15.15 h
Newark Castle					
Outside		178.0	3.8	79.8	8.1.98; 14h
Room 1	(centre)	1.2	3.8	81.2	
	(range)	(0.9-1.2)			
Room 2		0.2	4.2	85.0	
		(0.1-0.6)			
Room 3		0.3	4.0	82.5	
		(0.3-0.6)			

Non-continuous climatic measurements are not sufficient to investigate the overwintering site environment. Its climatic variations in relation to outside changes have to be examined to see to what extent a stable environment is provided. More importantly, it is the micro-environment in the crevices that has to be measured.

To that end, continuous records of temperature in a crevice in Room 2 of Newark Castle and outside the Castle (east facing) were obtained between 13 February 1998 and 15 March 1998. It was intended to record humidity as well but the high relative humidity inside the crevice and low ambient temperature meant that the humidity probe did not work as planned. Probably water condensed on the probe. Thus, humidity in the

crevices was measured at several occasions using the "wiggly wire" technique and the Vaisala humidity probe.

Table 6.3 Relative humidity in two overwintering sites

	Crevice RH (%) ± S.E.	Air RH (%)	Date
<u>Yellowcraig</u>			
Outside		80.9**	21.1.96
Cave	97.5 ± 0.4 *	80.7 **	
Cave	97.5 ± 0.2 *	84.5 **	28.1.97
<u>Newark Castle</u>			
Room 1 *	96.9 ± 0.3	95.6 ± 0.4	11.3.97
Room 2 *	96.8 ± 0.4	92.5 ± 1.1	(Fog)
Outside **		87.0	18.2.98
Room 1 **	95.1	87.5	
Room 2 **	98.3 ± 0.4		
Outside **		97.6	3.3.98
Room 1 **	97.7	98.2	
Room 2 **	97.6 ± 0.4	97.2	
Outside **		66.0	2.3.98
Room 2 **	93.7 ± 2.3		
Outside **		75.0	25.3.98
Room 2 **	95.5		
Outside **		73.0	1.4.98
Room 2 **	98.0		
Outside **		60.0	14.4.98
Room 2 **	86.0		

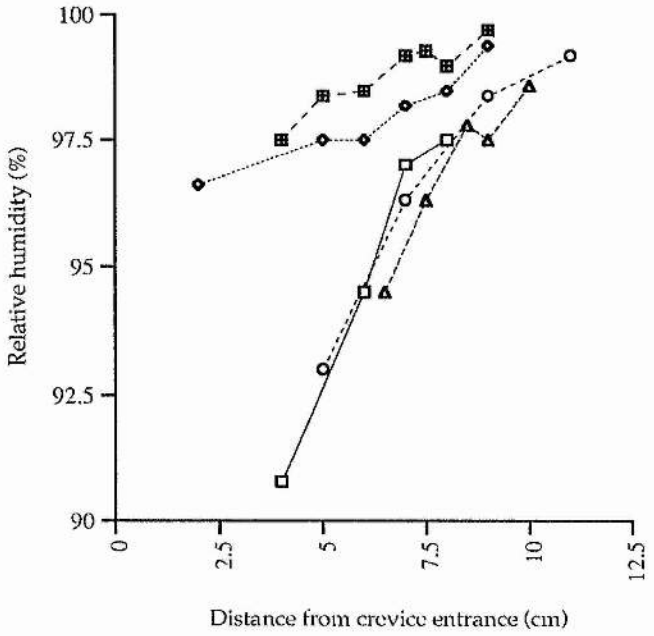
* Measured with "wiggly wire" technique
** Measured with Vaisala humidity probe

It is clear that throughout the overwintering period, humidity in the crevices is high, well above 90%. On 14 April 1998, when the lowest humidity was recorded, there was only one fly left in Room 2, thus overwintering was almost over. Humidity was visibly not constant in the sites (Yellowcraig and Newark Castle): sometimes the walls were rather dry, but on many occasions water was seen dripping from the walls and the roof. Again, outside variations in humidity are not reflected as strongly in the crevices. A high, fairly stable humidity is thus characteristic of the crevices where *E. tenax* were found to overwinter. A humidity gradient, getting higher at greater depth, was often recorded within the crevices, as shown on Figure 6.4.

Figure 6.5 shows temperature measurements. The first striking observation is that inside (in the crevice) temperature is much more stable than outside temperature. During this period, the outside temperature reached a minimum of 0.1 °C and a maximum of 15.1 °C and averaged 5.8 °C, whereas the crevice temperature varied between 8.6 and 13.0 °C with a mean of 10.3 °C. Outside temperature showed large daily variations with a peak in the morning, that is when the probe was exposed to the sun, and a minimum during the night. The temperature range of one day could be as much as 12 °C (for example on 9 March 1998). Crevice temperature, on the other hand, did not show a daily pattern of variation, even though it tended to be lower at night. The maximum range recorded for one day was 2 °C. It is therefore clear that temperature conditions in crevices are more stable and are not affected by large or rapid external changes. The average temperature is also higher (in the winter) than outside.

Light levels could be important for this fly as a trigger to the end of overwintering. It was noticed several times that flies started moving when light was shone on them, and some did fly away after a census. These overwintering syrphids do respond to light and can become active quickly when disturbed. As was seen in Chapter 6, they need to warm up endothermically before they can fly, but they can do so at temperatures as low as 5 °C, as was observed in Yellowcraig. However, it is doubtful if light is used by the flies to sense time because they were found to overwinter in quite variable sites regarding light. For example, Room 1 in

a/



b/

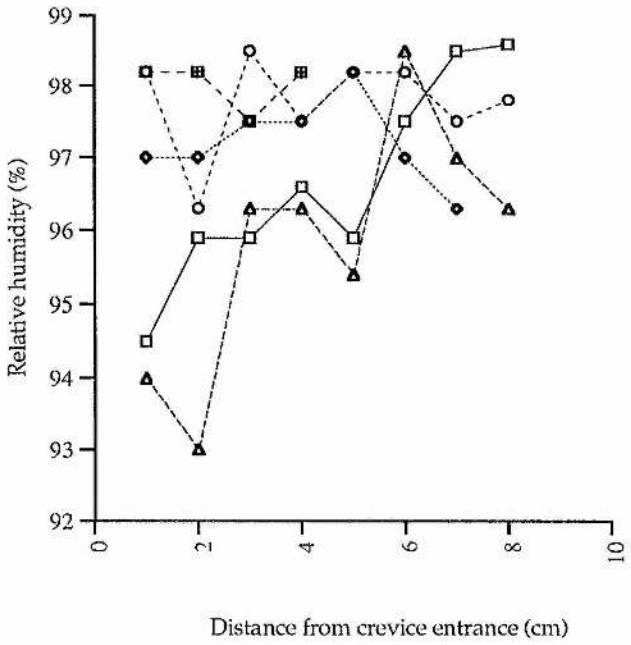


Fig. 6.4 Relative humidity measured inside crevices by the "wiggly wire" technique
a/ At Yellowcraig on 21.2.96
b/ At Newark Castle on 10.3.97

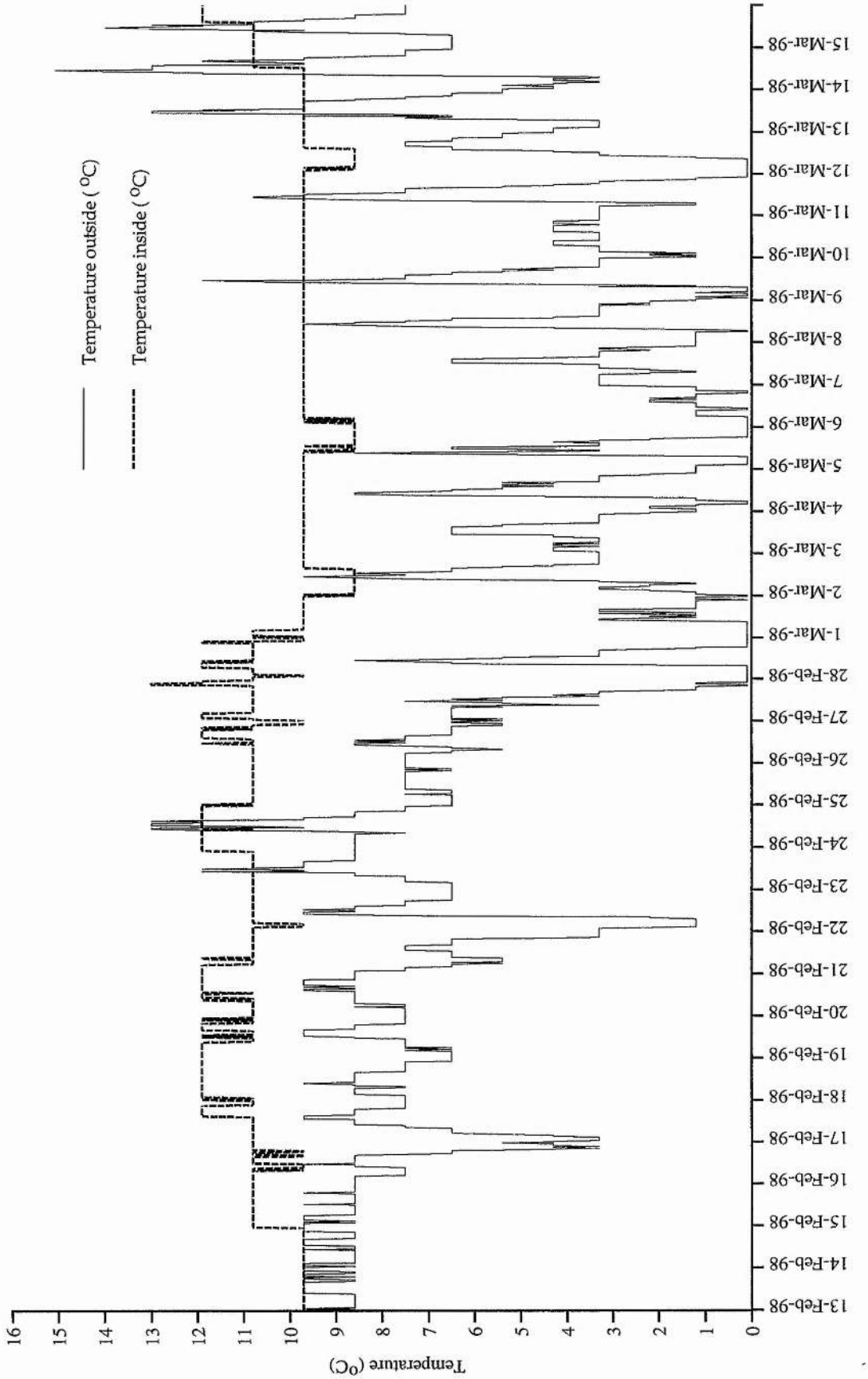


Fig. 6.5 Temperature records in one crevice of Room 2 and outside at Newark Castle during winter1998

Newark Castle receives light through two openings in its east wall and two openings in its west wall. On the other hand, Room 2 has only one opening in its west wall and is very dark (Table 6.2). Moreover, flies which are deep in narrow crevices are unlikely to receive much light. Nevertheless, crevices closer to openings were amongst the most occupied.

6.3.2 Site selection - Behaviour

The first autumnal visit to Newark Castle was on 17 September 1998. Several crevices were already occupied by female *E. tenax*.

E. tenax seem to use the same crevices every year. Some crevices adjacent to occupied ones were found empty on the three successive years (at Kilrenny and Yellowcraig). No obvious visible characteristic distinguished the empty and filled crevices, but the microclimate of such empty crevices would be worth measuring. The flies use quite differing crevices: some are very narrow and deep, whereas others are shallow and wide. The former probably offer a more stable microenvironment than the latter, but no occupancy preference was noted. Occupied crevices tend to be above one meter from the ground. *E. tenax* can overwinter alone or share a crevice with a few others not in close body contact with each other. Alternatively, they are also found highly clumped together, even on top of each other in some instances (Figures 6.6a, b, c & d). Typically, overwintering *E. tenax* have their head towards the inside and abdomen towards the outside of the crevices. However, they are sometimes looking out.

Several flies were observed looking for and selecting overwintering sites. Typically, the flies come from outside and start "hovering" along a wall. After a while, they enter a crevice and seem to check it. None of the flies observed remained in the first checked crevice, and all visited several, in various places (both east and west walls and roof). Some flies settled in already occupied crevices, whereas others used empty crevices. Some crevices which were occupied on 22 September 1998 were empty on 30 September 1998 (Figure 6.7 and 6.8). Thus in these early days, flies do not necessarily remain in a selected crevice. Such crevices might become occupied later on.

a/

b/

Fig. 6.6 *E. tenax* overwintering - flies in clusters in narrow crevices

c/

d/

Fig. 6.6 *E. tenax* overwintering
c/ Flies in a cluster in a shallow crevice
d/ Flies spread out in a shallow crevice

Marking overwintering flies turned out to be more difficult than thought because most of them fly way when disturbed. However, the procedure was successful in 11 instances. The marked flies did not move from one crevice to another in the same site. Some flies disappeared (left or were killed), and some carcasses were found.

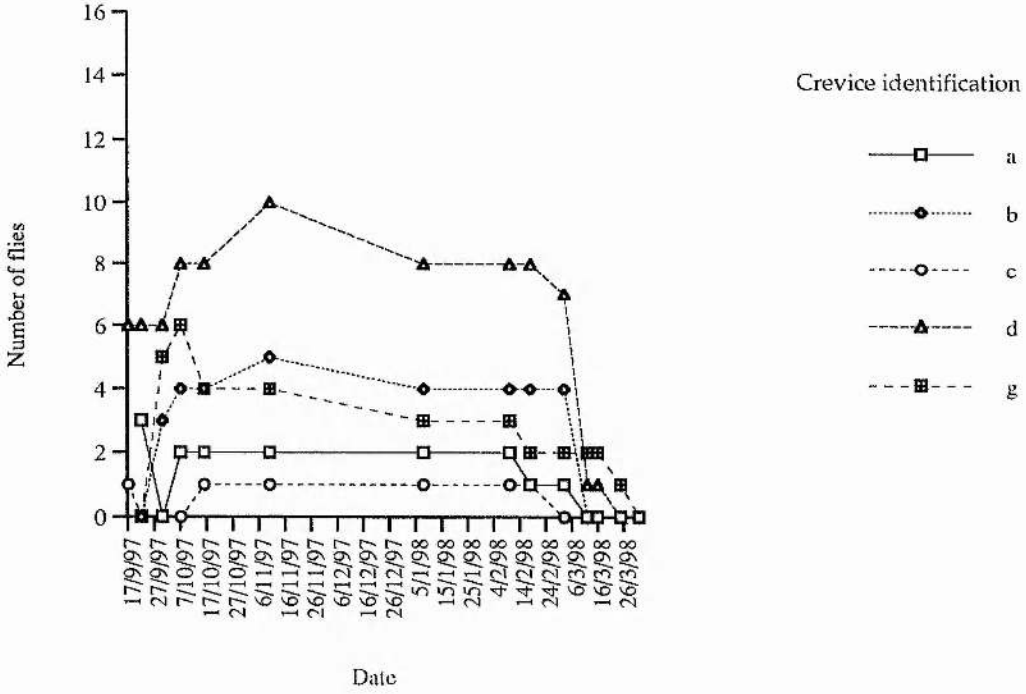
E. tenax do move in the crevices during overwintering. Some go more or less deep whereas others are clumped at times and spread out at others. Also, some flies were observed outside crevices. Towards the end of the overwintering period, they were often gone at the next visit, and it can be assumed that these were ready to fly away. Two flies, at Newark Castle, were repeatedly sighted outside crevices (in March 1997). In Yellowcraig, a few flies were seen outside crevices at each visit, but position recording and marking were not attempted as regular visits were not possible.

6.3.3 Variation in flies number and climate

This section refers to observations made during autumn 1997 and winter-spring 1998 at Newark Castle in Rooms 1 and 2 (Figure 7a, b, c & d and Figure 8a, b, c & d). Recording the number of flies overwintering was a tedious task, and the figures presented here are likely to be underestimates, as some crevices were probably overlooked and some flies missed out because they were too deep in the crevices. However, at each census, all the crevices previously recorded were inspected, so the variation in this set of crevices is a good reflection of changes in numbers. On 17 September 1998, overwintering drone flies were found in all three rooms, but Room 1 had more flies. The weather at that time was fine, still warm with moderate sunshine, but the temperature had started to decline (Figures 6.9 and 6.10). The flies had obviously started to overwinter before that date, but site searching behaviour was still observed: at that time, *E. tenax* was in the period of looking for overwintering sites. Several factors could trigger this behaviour, e.g. shortening of days and decrease in ambient temperature. The overwintering start is not a brief period, but extends over several weeks as, on fine days, *E. tenax* could often be seen feeding (usually on ivy until mid-November), and numbers in Newark Castle kept increasing until mid-November in certain crevices.

As mentioned above, early in the overwintering period the number of flies in each individual crevice is quite variable (Figures 6.7 and 6.8).

a/



b/

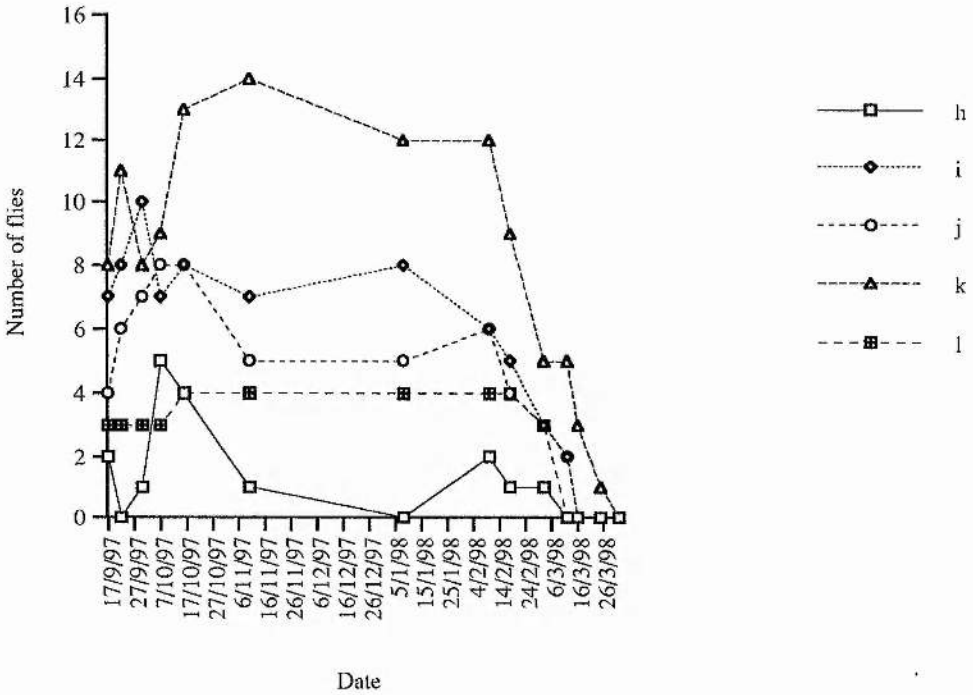
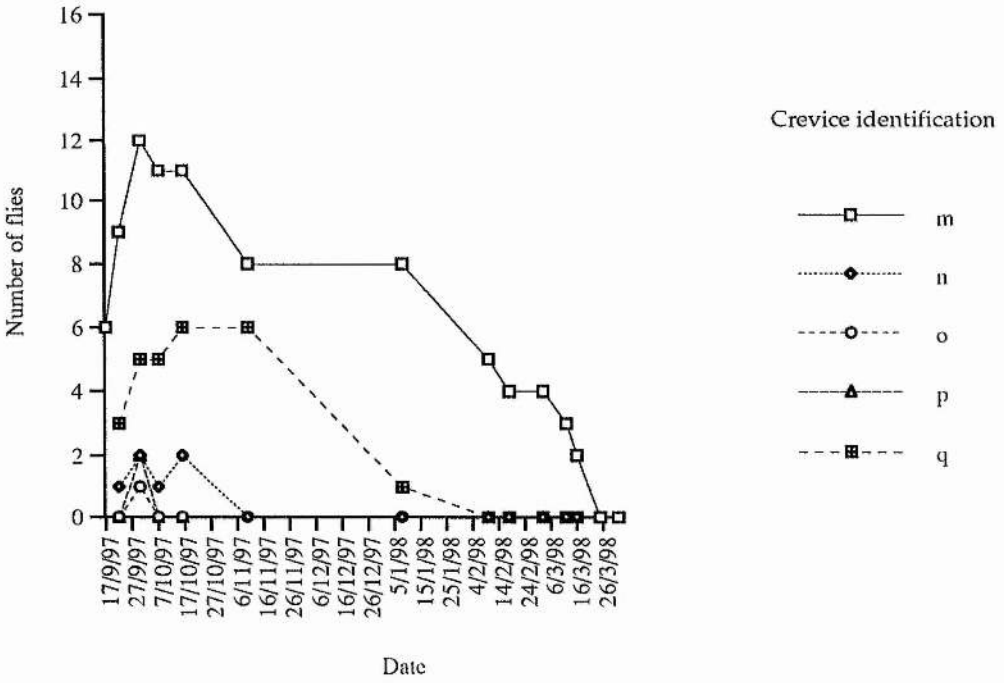


Fig. 6. 7a & b Variation in the number of *E. tenax* overwintering in crevices of Room 1 (Newark Castle) in autumn 1997 - winter 1998

c/



d/

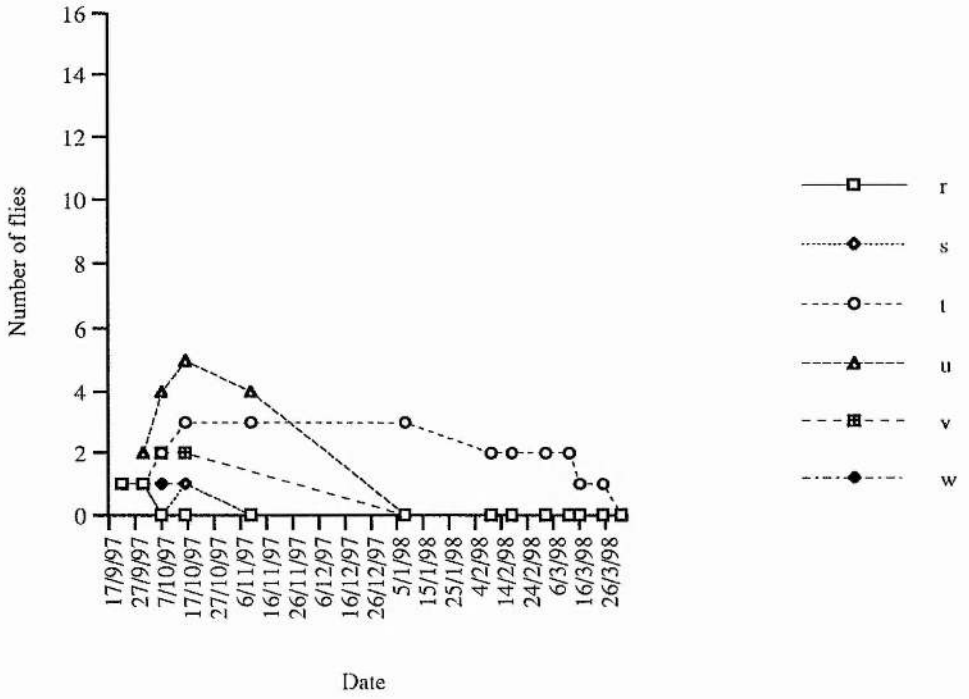
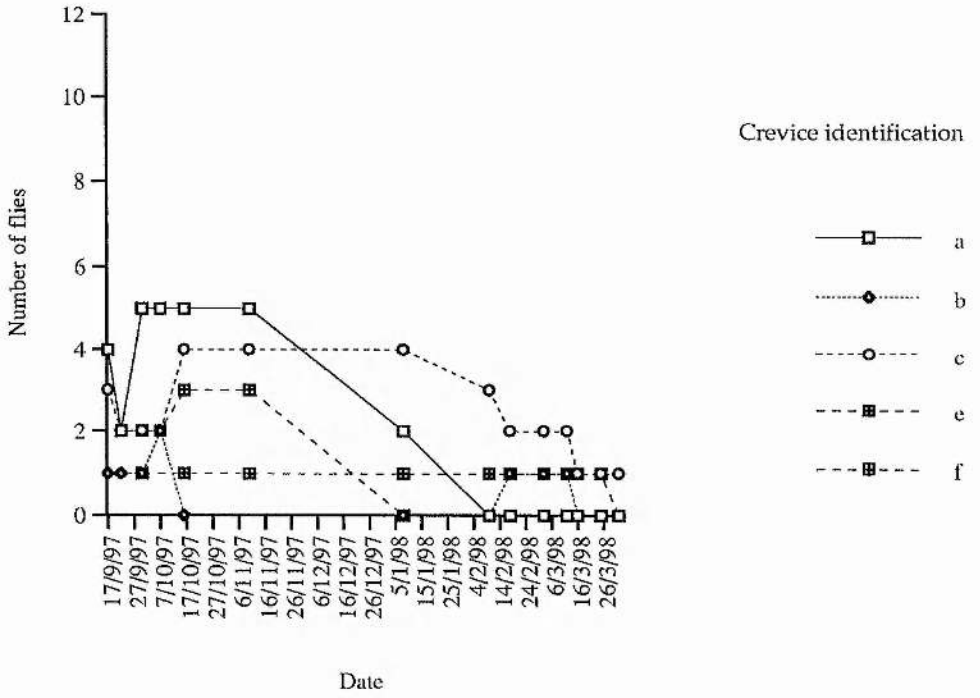


Fig. 6. 7c &d Variation in the number of *E. tenax* overwintering in crevices of Room 1 (Newark Castle) in autumn 1997 - winter 1998

a/



b/

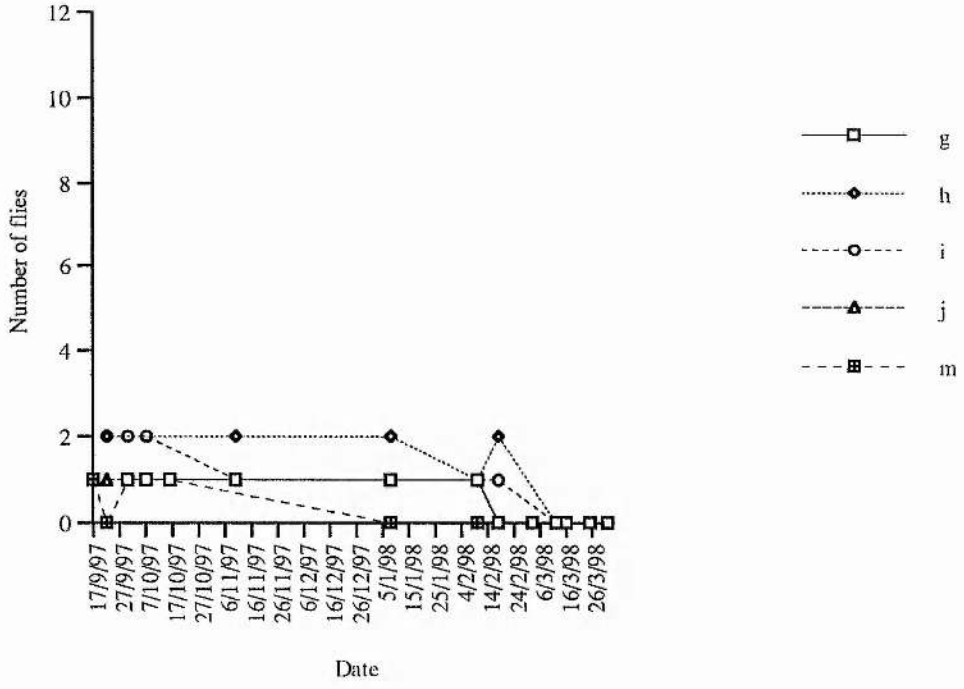


Fig. 6. 8a & b Variation in the number of *E. tenax* overwintering in crevices of Room 2 (Newark Castle) in autumn 1997 - winter 1998

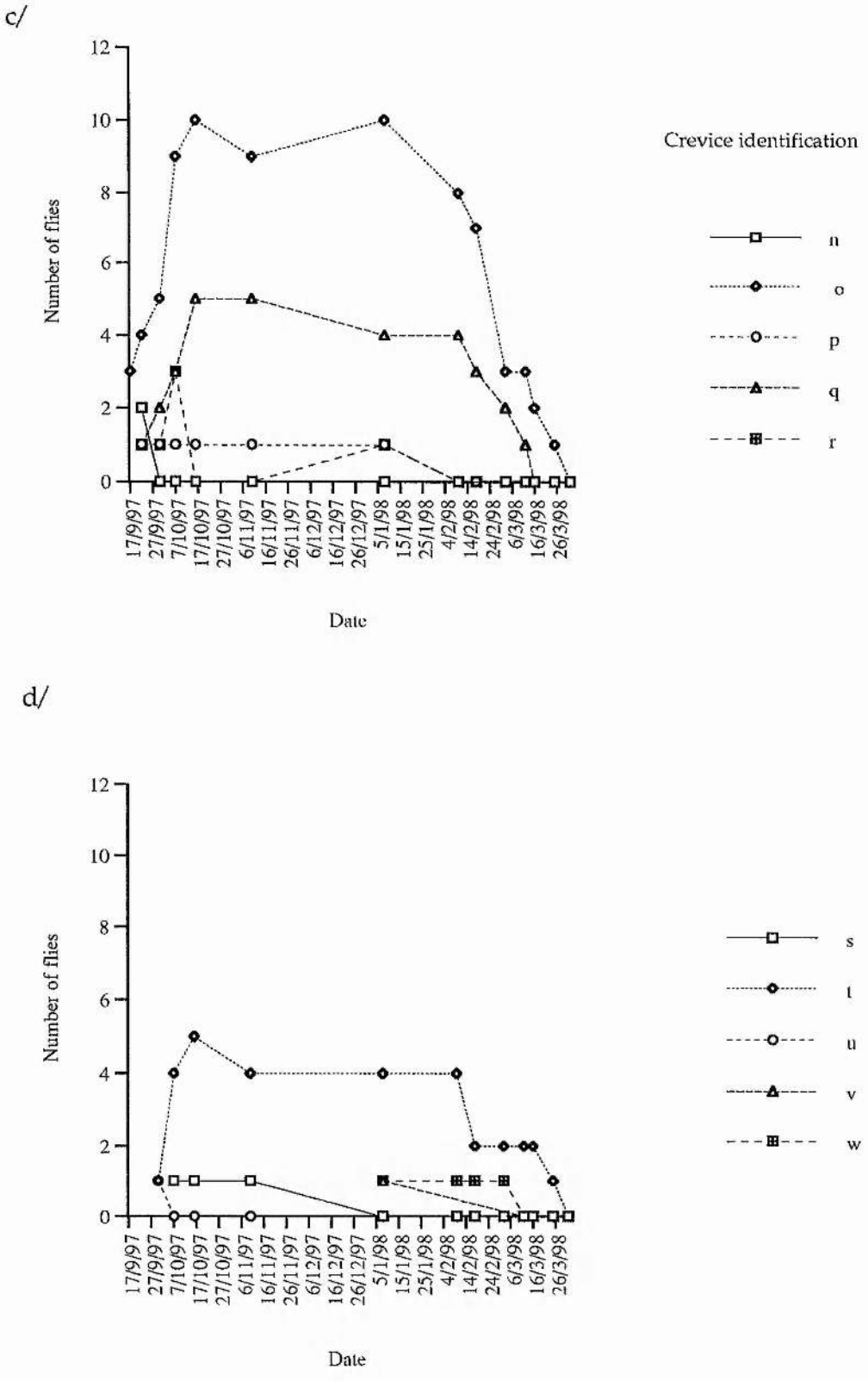


Fig. 6. 8c & d Variation in the number of *E. tenax* overwintering in crevices of Room2 (Newark Castle) in autumn 1997 - winter 1998

The fluctuations in counts were lessened by mid-October. Most crevices which were already occupied on 17 September sheltered clusters of flies (Figures 6.7 and 6.8). In some instances where there were one or two flies in a crevice (e.g. crevices n, o, p, r, s and w in Room 1 and crevices b, n and u in Room 2) the flies had left by early November (Figures 6.7 and 6.8). Thus, clusters seem to be favoured.

The maximum number of flies was recorded on 16 October 1998, but occupancy in some crevices kept increasing until mid-November. The period of increase in the number of flies settling in the overwintering site corresponds to a decrease in temperature (Figure 6.9 and 6.10). In August 1997, even though temperature varied a lot, the maximum day temperature never got below 14 °C and minimum night temperature remained above 10 °C. From September onwards, temperature started to decline markedly (Figures 6.9 and 6.10).

From mid-October to mid-February, the number of flies in Newark Castle decreased slowly but steadily. This suggests that some flies left the site during winter and/or that some died. Although in some crevices some counts showed an increase, these were usually preceded by a decrease (e.g. crevices i and j of Room 1). In Room 2, flies started being recorded in January in two crevices (Figure 6.7 and 6.8): either these crevices had been overlooked previously or some flies had moved in. Siuda (1963) also noted some increase in some crevices. As the flies could move very deep in the crevices, it is possible that some, even though they were in the crevice, were too deep to be seen. Some could also be hidden by irregularities in the stones. Moreover, counting the number of flies in a clump was difficult. As of the marked flies none was observed to move from one crevice to another, the most likely explanation for the variable recorded occupancy when overwintering was well under way is the inaccuracy of the counting process. More extensive marking could help to some extent to get a better idea of possible movement between crevices. It is however impossible to eliminate the possibility of immigration, emigration or limited movement between the crevices. In any case, Figures 6.7 and 6.8 show that variations are quite limited.

The number of flies overwintering started to drop sharply from mid-February. This corresponded to a substantial rise in temperatures. Indeed, 13 February 1998 was recorded as the warmest day in February in Britain

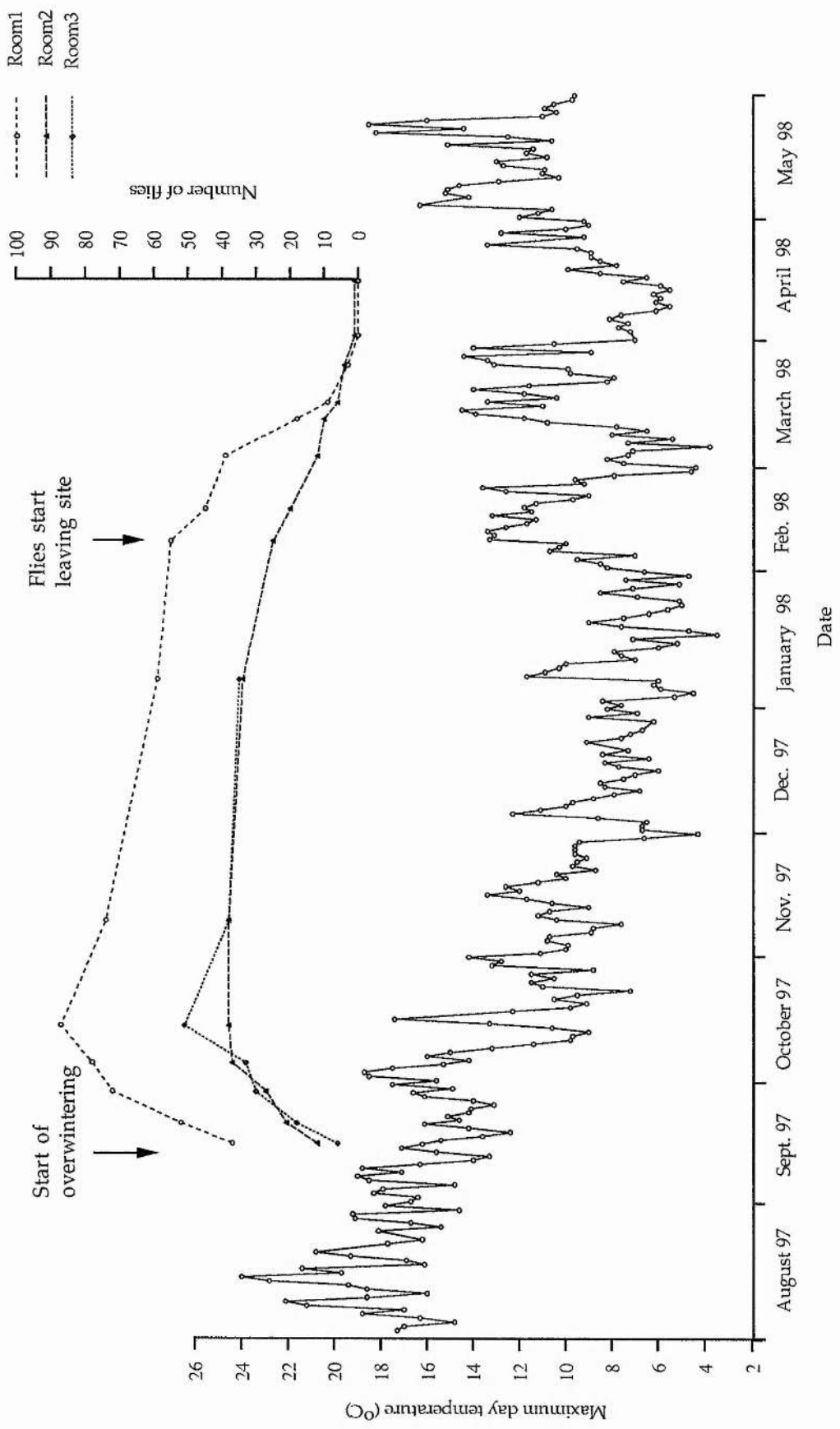


Fig. 6. 9 Overwintering females *E. tenax* in relation to maximum day temperature. Newark castle. Autumn 1997- Winter 1998

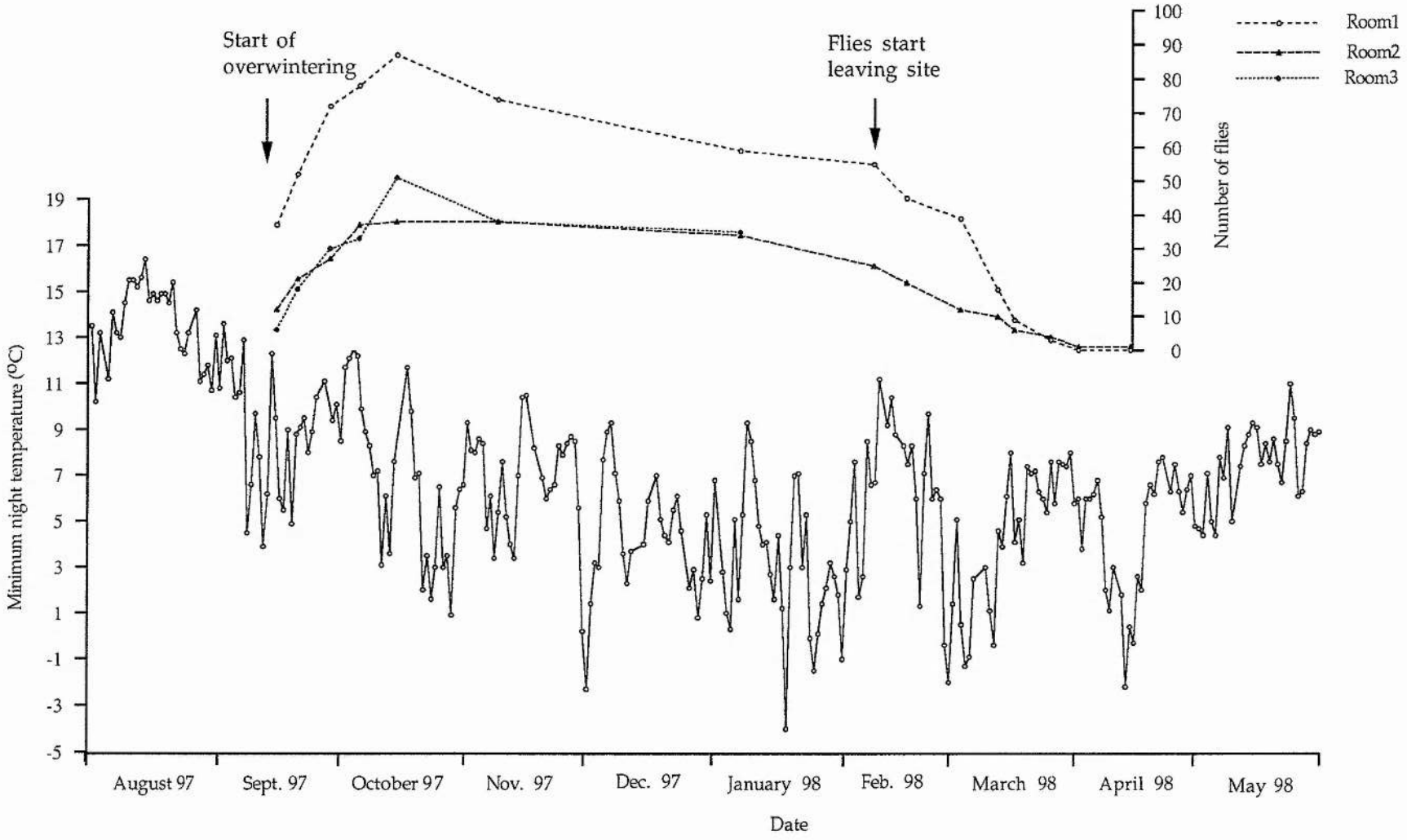


Fig 6. 10 Overwintering females *E. tenax* in relation to minimum night temperature. Newark castle. Autumn 1997- Winter 1998

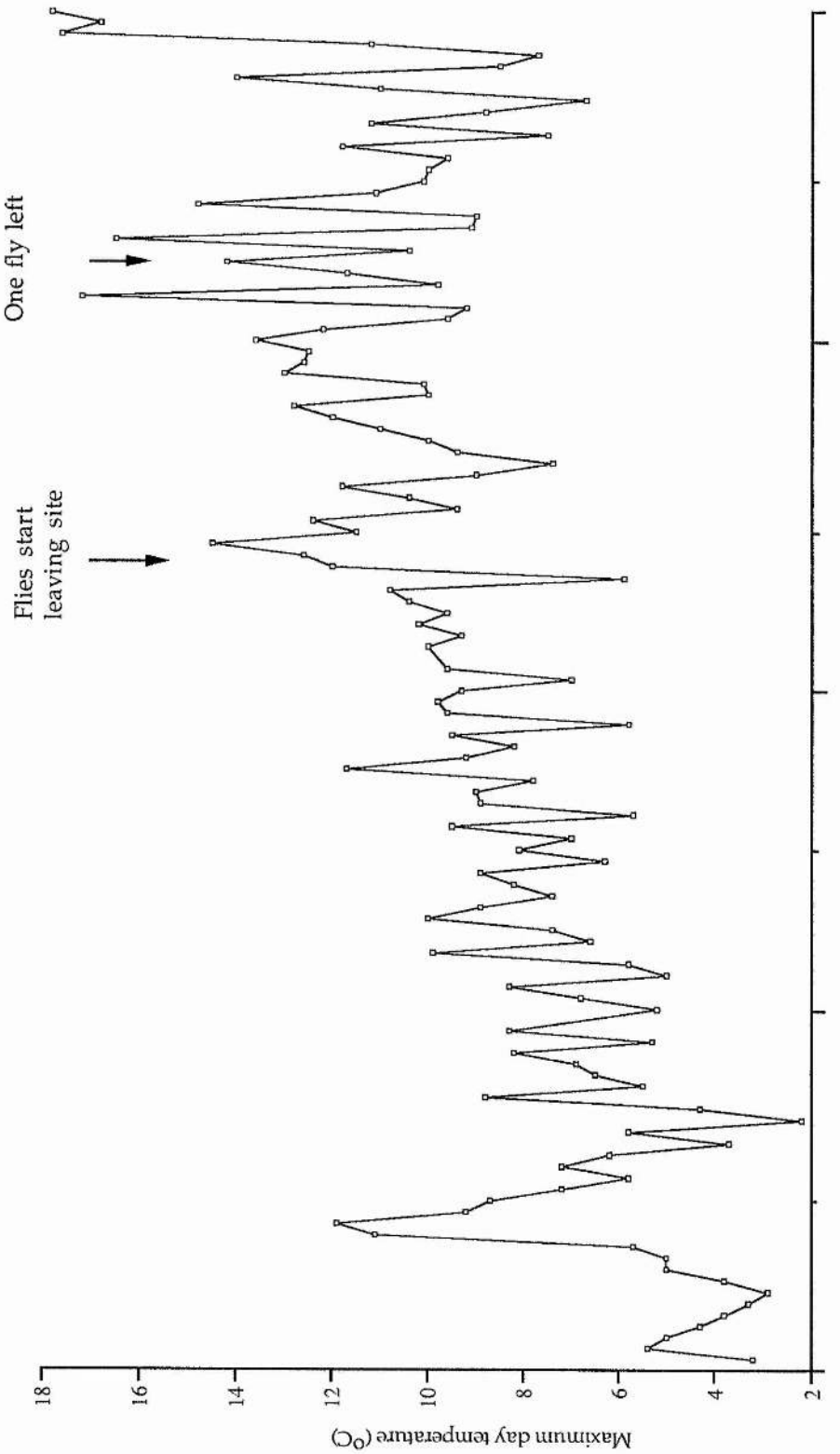


Fig 6. 11 Overwintering females *E. tenax* in relation to maximum day temperature. Newark castle. Winter 1997

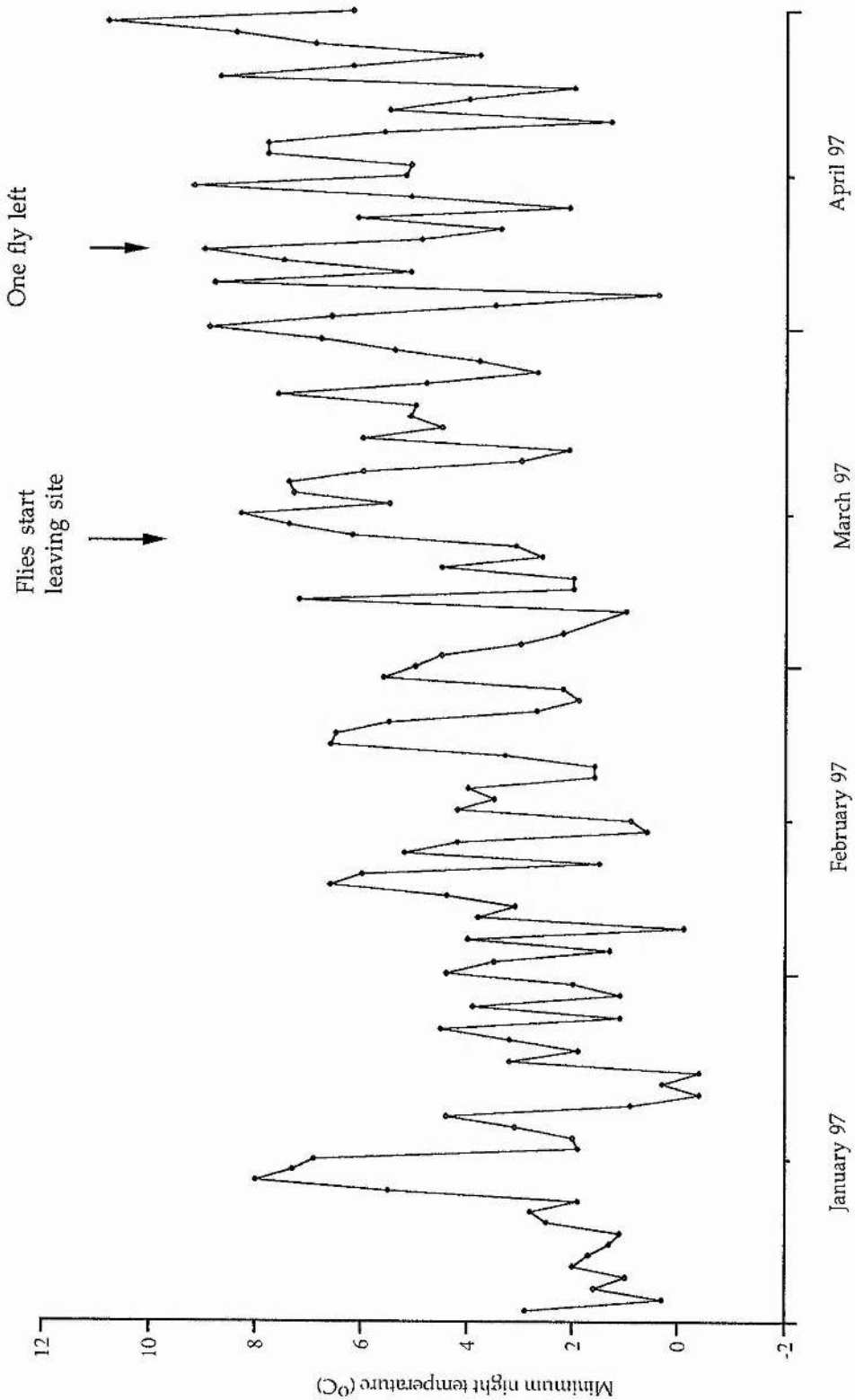


Fig 6. 12 Overwintering females *E. tenax* in relation to minimum night temperature. Newark Castle. Winter 1997

since records began; temperature reached 17.5 °C at Banchory, Grampian (The Scotsman 14 February 1998). At Fife Ness, temperature was as high as 13.6 °C. This period of warm weather lasted from 8 to 25 February with some cold spells on 16, 21 and 22 February (The Scotsman 24 March 1998, Figures 6.9 and 6.10). In mid-March/April some female *E. tenax* were sighted feeding on lesser celandine (*Ranunculus ficaria*) and dandelion (*Taraxacum officinale*) around Newark Castle.

This exceptionally warm weather worried animal experts because animals like hedgehogs were likely to come out of hibernation and find themselves without food and at risk because of possible subsequent cold weather (The Scotsman 14 February 1998). There is little doubt that it also triggered *E. tenax* arousal. Similarly, in 1997, although Newark Castle was not studied in such detail, it was noted that the flies started to leave the site around mid-March, and all had gone by 8 April; there had been some fine warm days during that interval (Fig 6.11 and 6.12). The "trigger" outside temperature seem to be around 13 °C, but it is likely that a persistent high temperature (i.e. for a few days at least) is required. Incidentally, the bats which were hibernating in Newark Castle (3 in 1997 and 2 in 1998) left around the same time as the flies.

6.4 Discussion

This part of the project is essentially just a pilot study. A suitable overwintering site allowing frequent visits was only discovered during the second winter. Thus, a more comprehensive study could only be carried out in the third winter; the data from the two preceding winters are more sporadic. As a consequence, no statistical analysis would be meaningful. Nevertheless, much can be learned from the physical and climatic characteristics of the overwintering sites, and suggestions can be made about the behaviour of *E. tenax* during the overwintering period. From the information gained, further studies could be planned in order to verify these suggestions.

6.4.1 Choice of overwintering sites

All the overwintering sites of this project were made of rock/stone. There have been some reports of *E. tenax* spending the winter in wooden outhouses in England (Willmer, pers. comm.), but various enquiries failed to confirm that this also happens in Scotland. It would be interesting to investigate further this issue, as the microclimate provided, especially as far as humidity is concerned, is likely to be different from the sites reported here. It is possible that humidity is not such an issue in the south because the flies might have more opportunities to leave the site to collect food and water; certainly *E. tenax* has been sighted many times in winter (Kato 1943, Hastings 1988, Gilbert pers. comm.). Hastings (1988) recorded some female *E. tenax* (dark form) from 22 December 1987 to 23 February 1988 which was an exceptionally mild winter. The flies were found particularly on *Viburnum* spp. or resting on sunlit leaves in Kew gardens. Kew Gardens offer a well protected site from the weather, and being in London probably benefit from some extra heat.

The various sites identified for this project provide a high, quite stable humidity as well as temperatures which are not as affected by the wide fluctuations of air temperature and which remain well above freezing. Kendall and Stradling (1972) also reported that *E. tenax* overwinter in damp places which must not be prone to flooding. Moog and Christian (1978) and Siuda (1963) recognised that the cave environment provides a fairly stable climatic environment. A high humidity is certainly important for overwintering *E. tenax*. In Chapter 3, it was demonstrated that these flies are losing water at a rate which falls in the upper part of the range for Diptera and other mesic insects. It was also suggested that they become quite dehydrated during overwintering and that they do lower their rate of water loss during that period. All this indicates that overwintering drone flies are water stressed. It therefore makes sense for them to select a site which provides a high humidity so that water losses can be limited. Forming a cluster can also be of some help by reducing the effective surface area to volume ratio. This could explain the occurrence of clusters even in crevices where the flies could be spread out. However, some flies are alone in a crevice or share a crevice with others but remain spread out. There could be some competition to be included in a cluster as the first crevices to be occupied sheltered clusters

of *E. tenax*. Alternatively, some crevices might provide a more favourable microclimate than others. Perhaps in these, the drone fly does not need to form clusters. It would be informative to investigate the microclimate offered by particular crevices in relation to the occurrence of clusters. A humidity gradient was measured in several crevices (Figure 6.4), and it is possible that the flies use this gradient to help maintain their water balance. Certainly, some movement in the crevices was noted, with the flies being sometimes very deep in a crevice and sometimes very close to the entrance.

Continuous recording of a crevice temperature confirmed the temperature stability offered by the overwintering site. Even when outside temperature was close to freezing, the crevice temperature never got lower than 8.6 °C. The average crevice temperature was almost 5 °C above average outside temperature from mid-February to mid-March. The crevice temperature does vary but much more slowly than outside temperature: variations are dampened. The crevices are protected from the wind and do not receive any sunshine: daily variations are much smaller (- 2°C maximum compared to 12 °C maximum outside). In addition, the air of the cave or room offers some thermal damping. Air temperature in the caves is closer to outside air temperature, but because of the protection from the wind and sunshine it is bound to change more slowly. Siuda (1963) also reports that cave temperature is above outside temperature in winter. It would be worth having a third data logger recording cave air temperature simultaneously with outside and crevice air temperature. The only data available here are non-continuous records. For example, on 18 February 1998 at 10.00 am, outside temperature was 9.3 °C, Room 2 temperature was 9.0 °C and crevice temperature (in Room 2) was about 12 °C; on 3 March 1998 at 10.30 am, outside temperature was 6.9 °C, Room 2 temperature was 5.5 °C and crevice temperature was 9.8 °C. Outside temperatures were measured in the sun, which explains why they are higher than room temperatures, which had only started to increase from the low temperatures experienced during the night. Kendall and Stradling (1972) report that they did not find any overwintering drone flies in crevices on exposed rocks. They propose the risk of flooding as an explanation for the lack of flies, but the less stable microenvironment that would be provided in these crevices could also play a part. Indeed,

searches made for this project in crevices that would not be liable to flooding but were more exposed (such as on the outside walls of Newark Castle) failed to find any overwintering fly. Thus, the crevices being situated inside caves or buildings provide extra protection from thermal instability and cold.

Overwintering flies do not risk freezing. It is thus likely that they do not need any particular physiological adaptation, such as the production of anti-freeze, to survive the winter. In addition, as temperature does not fall very low (it remained above 8.5 °C during the studied period), the flies can become active very easily. These are temperatures at which they can walk and can warm up endothermically to fly (lowest air temperature at which *E. tenax* was seen to fly is 5.0 °C). It is possible that they go deeper in crevices in response to cold. The verification of this suggestion would require the investigation of the presence of thermal gradients within the crevices and of fly movement in response to changes in temperature. Again, forming clusters increases thermal mass and could assist in the protection against cold. As for humidity, it would be interesting to compare the thermal stability of crevices where clusters are formed to that of crevices where no clusters were found.

The importance of light in the overwintering site is more difficult to determine. Siuda (1963) and Moog and Christian (1978) report that *E. tenax* occupy crevices close to the entrance of caves or mines where light is available. Siuda (1963) found *E. tenax* in areas having light levels (measured at mid-day with average overcast) ranging from 0.04 to 0.4 W m^{-2}. In this project, flies were found in crevices which did not receive much light, as for example in Room 2 of Newark Castle. The south wall of Room 2 received only about 0.1 W m^{-2} during the day. In addition, light levels deep in crevices are probably very low. However, it is true that more flies were recorded at the entrance of the cave at Yellowcraig and on the north wall of Room 2 (the one which receives more light) and close to openings in the 3 rooms of Newark Castle. Thus, it appears that light must have some importance to these flies. It might be used to determine when to leave the overwintering site but probably in association with other factors such as temperature.

6.4.2 Initiation and termination of overwintering

The results suggest that temperature has an important role in triggering the start and the end of overwintering. As mentioned above, light could also be a trigger, with decreasing photoperiod prompting overwintering and increasing photoperiod signalling the time to leave the site (Davenport 1992, Moog and Ernst 1978, Siuda 1963,). However, the results suggest that light does not operate on its own as a trigger, because on two successive years *E. tenax* terminated overwintering in Newark Castle with a month difference. It is however possible that both temperature and light operate together. Davenport (1992) suggests that temperature can also affect overwintering. Using light as a second stimulus would prevent the flies from leaving the site too early if the temperature happened to be high enough. It is also possible that the preference of the flies for crevices close to light sources merely reflects the need to know when it is day and night before leaving the site. Certainly, the effect of photoperiod on triggering the end of overwintering should be investigated more thoroughly, possibly by manipulating the light received by certain crevices.

It is unlikely that humidity could trigger overwintering as it does not show a steady change at different seasons. It depends too much on daily weather such as rain, fog or sun. It is doubtful if it could influence the end of overwintering as records show that humidity in the crevices was still very high when the flies were leaving.

Therefore, temperature seems a good candidate as the primary trigger for overwintering. As temperature declines at the end of summer, early September in 1997, female E. *tenax* start looking for sites to spend the winter. The number of flies increased in some crevices until mid-November 1997, but total numbers reached a maximum by mid-October in Newark Castle. Siuda (1963) reports that, in his study, numbers increased form October to December 1959, quite similar findings to the present ones. Although there is a decline in numbers throughout the overwintering period the sharp decrease, which signifies the end of overwintering, corresponds to an increase in temperature. It is likely, that *E. tenax* will not leave the site if temperature is higher for only a short time (e.g. daily variations). A more sustained rise should be necessary to prevent the flies coming out and getting stranded in cold weather. This is probably

achieved because the temperature in the crevices responds slowly to outside changes and will only start rising if outside temperature remains high for some time. The slow response of crevice temperature is illustrated on Figure 6.5. Crevice temperature started to decline about a day and a half later than outside temperature at the end of February 1997, and it took about the same time to start increasing in mid-March. At outside temperature around 13 °C (crevice temperature around 12 °C), *E. tenax* started leaving the site. Although they can fly at lower temperature, this is a temperature at which most *E. tenax* will warm up endothermically and fly readily, as was shown in Chapter 5 (in the laboratory and in the field). In Siuda's study (1963), *E. tenax* numbers decreased sharply around end of February/mid-March 1960. This also corresponded to an increase in temperature, although temperatures around 5 °C seem sufficient to have triggered leaving. It would be interesting to carry out a comparative study of the thermal physiology of *E. tenax* from two such differing climatic regions as Scotland and Poland. Average temperature goes down as low as - 21 °C in winter in Poland, and although caves offer some protection, their temperature also goes below freezing.

Relying on temperature as a stimulus to end overwintering carries the risk of leaving too early. In 1997, *E. tenax* started leaving around mid-March; subsequent temperatures remained high. However, in 1998 the exodus started in mid-February. Temperatures got cold again at the end of February and at the beginning of April. When they leave the overwintering sites, the females are unlikely to lay their eggs immediately. Kendall and Stradling (1972) found that between eight and fifteen days after overwintering was terminated were necessary for egg maturation. Female *E. tenax* leaving overwintering sites will thus need to find some food and be able to survive at least a week before they can lay their eggs. If they come out too early, they might not find the necessary food or they might get stranded if cold weather returns. They do not come back (or very few do) to their shelter after they leave (numbers keep decreasing). Moreover, Mood and Christian (1978) report that no *E. tenax* was sighted in the mine they studied after April and during summer months. Also, it might not be a good strategy to lay the eggs if temperatures are likely to become very cold again. It is not possible to say how *E. tenax* were affected by this early start in 1998. Numbers remained low throughout spring and

by this early start in 1998. Numbers remained low throughout spring and summer, but the weather during summer 1998 was particularly bad. Therefore, the concerns that animal specialists showed for hibernating hedgehogs in February 1998 (The Scotsman 14 February 1998) could well also apply to *E. tenax* which might have been lured out of overwintering too early by a few days of unseasonable weather.

The various reports of *E. tenax* feeding in the winter mentioned above suggest that this fly is also active in winter in some regions like the south of England. A detailed study of overwintering in southern England could reveal differences from Scotland. It is possible that with the weather being milder, especially in cities, overwintering is shortened. However, Siuda (1963) reports sighting *E. tenax* resting on limestone and flying short distances in the "warm" day of January 1957, in Poland; drone flies in Poland might be better adapted to cope with cold weather. In Fife (Scotland), I have never seen *E. tenax* flying and/or feeding in December or January. Anne Reid (Dundee Naturalists' society, Scotland) (pers. comm.) observed female drone flies feeding on crocus in her garden on 6 March 1997 at a temperature of 7-8 °C. This site is quite sheltered. The earliest I have seen *E. tenax* active is on 8 April 1997, when females were feeding on lesser celandine at Newark Castle (ambient temperature in the sun 11.5 °C). On 14 April 1998, one female *E. tenax* (and one male *E. pertinax*) were seen feeding on dandelion at Newark Castle (ambient temperature 6.1 °C). It is unlikely, but not impossible, that these early drone flies came from a new generation; at least 5 weeks are needed for development from eggs (and this duration is temperature dependent) (Heal 1979a) and eggs are laid eight to fifteen days after the end of overwintering (Kendall and Stradling 1972). It is therefore more likely that these were females which had overwintered.

6.4.3 Behaviour during overwintering

At the beginning of overwintering, the number of flies in particular crevices goes up and down (Figures 6.7 and 6.8) whereas the total number of flies increases. Moreover, it was observed that at this time, flies do not necessarily stay in the crevice they first entered. They seem to try out several crevices before settling down. What cues they are picking is

uncertain, but probably the suitability of the crevice in providing a well sheltered habitat is assessed.

By mid-October flies start settling down, and numbers stop increasing by mid-November. This corresponds to the time at which the last flies are seen feeding in the field. From then to the time *E. tenax* leave the overwintering site, numbers decline steadily (from 87 to 55, early February, in Room 1 and from 38 to 25 in Room 2). Moog and Christian (1978) and Siuda (1963) also observed such a decrease. They attributed it to flies' death. Certainly some die as numerous moulded carcasses were found; some are also killed by spiders. Marking did not suggest any movement of flies between crevices at this time, but this should be investigated further with more flies marked. However, the decline cannot all be attributed with certainty to death. This would leave the possibility that some flies leave the site early. As has already been mentioned, some active flies have been reported in England in December (Hastlings 1988, Gilbert pers. comm.), though none has been seen during the coldest months so far in Scotland. Nevertheless, it is possible that some *E. tenax* are forced to leave their overwintering site early, maybe because their water reserves are running low. These could find shelter in other sites or maybe come back to the same site (which would explain the few new flies recorded in some crevices). When disturbed, *E. tenax* can become active, warm up endothermically and fly away: they are therefore capable of leaving. This could explain the presence of some drone flies outside crevices. In any case, if flies do leave early it must be a very low number that do so.

This Chapter has demonstrated that *E. tenax* select sites which provide fairly stable, high humidity and temperature. Within the crevices of caves and ruins, the drone fly is well sheltered from the harshness of the weather outside. This habitat selection complements the physiological decrease of the rate of water loss exhibited by females in winter and helps in maintaining the water balance. *E. tenax* tend to overwinter in clusters in crevices close to light sources. The clusters could help further in reducing water loss and withstanding cold.

A certain number of deaths certainly occurs during winter. Movement of flies in and out of the site could not be confirmed but is possible, and could explain some of the apparent disappearances.

Temperature, perhaps in association with changes in photoperiod, seems to be the trigger for the start and end of overwintering. When temperature decreases in autumn, the drone fly starts overwintering; it comes out during periods of warmth in early spring.

Chapter 7 - Behavioural ecology

7.1 Introduction

In this chapter, three behaviours exhibited by *E. tenax* and *E. pertinax* in summer will be investigated: hovering by males; foraging and flower handling by both males and females. The aim is to see how climatic factors, and in particular temperature, are influencing these activities. The findings of Chapters 3, 4 and 5 suggest that these eristalines are likely to be affected by hygrothermal constraints. After preliminary analyses of the results, it looked unlikely that humidity would have a direct effect on the flies' activity. Also, Scotland is not reputed to be very dry, and water is unlikely to be a rare resource. Thus, analyses concentrated mostly on the effect of temperature, light and the time of the day.

Hovering is observed very frequently in early summer in male *E. pertinax*, but is almost absent in male *E. tenax* in Scotland. Surprisingly, Wellington and Fitzpatrick (1981) reported that *E. tenax* hover very commonly in spring in Canada. This is one of the behaviours related to mating and may (Wellington and Fitzpatrick 1981) or may not (Smith et al 1994) involve territory defence. Various other behaviours can be used for territory defence, such as patrolling, or defence of oviposition sites (Maier and Waldbauer 1979). Fitzpatrick and Wellington (1982) give a comprehensive review of insect territoriality which is not mentioned in great depth here as the present work is more concerned with the climatic effects on hovering. Nevertheless they confirm that this behaviour is most probably linked to reproductive encounters, with males waiting and looking for passing females that they then pursue in order to mate.

When hoverflies chase passing insects to intercept conspecific females, they do not follow the passing insect but rather take an interception trajectory (Collett and Land 1978). If the passing insect is not

a receptive conspecific female, the hoverfly then returns to the same position as before. Collett and Land (1975) showed that to this end *Eristalis* spp. use visual cues from their environment.

Hovering is certainly very energetically demanding; in particular it was shown in Chapter 5 that a constant thoracic temperature around 30 °C is maintained in *E. pertinax*. The maintenance of a constant elevated thoracic temperature is well reported in other hovering flies (e.g. Smith et al 1994, Schutz and Gaugler 1992). It is likely that this behaviour will be constrained by climatic factors.

Several authors have reported that male hovering activity is timed to correspond with females' activity (e.g. Alcock 1996, working on carpenter bees; Maier and Waldbauer 1979, with syrphids). It is to be expected that if this behaviour is energetically highly demanding and if a male is to maximise its reproductive success, it would be to its advantage to hover when (and where) females are likely to be encountered.

Numerous studies (e.g. Herrera 1988 and 1990, Gilbert 1985, Willmer 1982b, 1983, 1985 and 1986) have investigated the influence of climate and food resources on foraging insects. In some studies, the thermal constraints faced by insects seem to prevail (Herrera 1990, Gilbert 1985, Willmer 1982b, 1983). Size, reflectance and endothermic abilities are all important. In other studies, especially in arid regions, humidity has some influence. It is often difficult to decide if a particular foraging pattern is adapted to available food rewards or if flowers have adapted their offering of food to the patterns exhibited by the foragers (Willmer 1983). In some cases it is not possible to disentangle these. For example, in the middle of the day temperature is usually at its highest point, as is light, humidity is at its lowest, and in these conditions low volumes of very concentrated nectar are available. Some insects peak in number at this time; this could be because of hygrothermal constraints, or to get as much energy per volume of food ingested, or both. On the other hand, it is clear that endothermic insects that forage early in the morning when nectar is often freshly secreted probably expend the necessary energy in warm-up in order to reach large quantities of nectar that other ectothermic insects cannot get. However, some insects select flowers according to their hygrothermal needs. For example, Willmer (1986) showed that the bee

Chalicodoma sicula switches to a plant that provides more dilute nectar when water stressed.

The present study looked at the foraging pattern of *E. tenax* and *E. pertinax* on oregano (*Origanum marjorana*) and to some extent aster (*Aster pyranacus*) in relation to food rewards and climatic constraints. Hoverflies, although secondary to bees as pollinators, are nevertheless important visitors for flowers (Herrera 1987, 1988, 1990; Jarlan et al 1997), but pollination was not investigated in the present work. The aim here was rather to determine the extent of the influence of the climate on these syrphids and to estimate the nectar reward they get. It has been demonstrated in Chapter 5 that both species are capable of endothermy, although only *E. tenax* thermoregulates in flight. Do they, as a result, forage independently of temperature? Do they, like bumblebees, forage in the early morning to maximise the volume (and/or caloric value) of nectar they collect? This study tried to answer such questions.

The time needed to handle flowers (time spent per flower by an insect to collect the nectar) is also likely to be affected by climatic factors. First, nectar availability and concentration are under the strong influence of temperature and humidity (as well as flower type). Nectar concentration increases and nectar volume decreases as water evaporates when ambient temperature rises (Willmer 1983, Proctor et al 1996). These climatic effects on nectar are stronger in open flowers, such as umbilifers, than in flowers with deep corollas, such as labiates (Willmer 1983, Proctor et al 1996). Insect agility and visitation also depend on climatic factors. As temperature and/or solar radiation increase insects' abundance rises (up to a critical temperature), and insects become more agile. This increased visitation depletes the flower nectar. Therefore, climatic factors, nectar availability and concentration and insects' abundance are all mutually dependent, and it is difficult to predict their precise effect on handling time. It would be expected that insects face constraints regarding handling time. Obviously, the reward has to be sufficient to justify the energy spent to get to the flowers. A large source of nectar would provide plenty of food. However, a large volume of nectar is likely to be dilute nectar (that is, freshly secreted nectar containing plenty of water), not bringing much energy, but heavy to carry. In addition, it would take a long time to drink

that nectar, during which the insect is exposed to predation (wasp predation on *Eristalis* was observed several times at the foraging sites). A smaller more concentrated nectar source is likely to be more valuable, as it would bring more energy, would be lighter to carry, and would require less time to drink as long as its concentration were not so high as to limit drinking speed. A highly concentrated nectar should not be favoured either because it will take longer to collect (if it is collectable at all) (Willmer 1983). Sotavalta et al (1962) demonstrated the decrease in drinking speed of flies as sugar solution concentration rises. Nectar feeders therefore face several trade-offs regarding nectar collecting. They should try to collect the most energetically rewarding nectar as fast as possible. In this work a preliminary study of flower handling time by *E. tenax* and *E. pertinax* was carried out to examine the influence of the climate and nectar characteristics on the drinking speed of these flies.

7.2 Materials and methods

7.2.1 *E. pertinax* hovering

This experiment was carried out in St Andrews Botanic Garden at the end of May, beginning of June 1997. A single fly was observed at any one time. Figure 7.1 shows a hovering male *E. pertinax*. With a stopwatch, the duration of hovering and resting was measured. Hovering was interrupted by periods of rest and/or basking. For each fly, the duration of the sequence of hovering/resting was recorded until the fly was lost from sight, for example when it flew away, or after a representative period had elapsed (around 10 minutes). Therefore for each period of recording, several measures for hovering and resting were obtained. Several of such records were obtained per half hour and grouped. For each half hour, the mean duration of hovering and resting was calculated, and the maximum duration of a single bout of hovering was also noted. The proportion of hovering relative to the period of observation was calculated.

Climatic conditions were recorded at the beginning of every half hour recording period and were assumed to remain constant during that time. Air temperature (T_a) and relative humidity (RH) were measured

a/

b/

Fig. 7.1 Male *E. pertinax* hovering

with a shaded hand held temperature humidity meter HMI 31 (Vaisala Ltd, UK) held about 2 m above the ground. Light levels were obtained from a hand held light meter (LX-101, Lutron) parallel to ground. Wind speed was measured from a hand held anemometer (Testovent 4000, Testoterm Ltd, UK) held about 2 m above the ground.

An attempt was made to mark the flies by catching them with a hand net, anaesthetising them briefly with CO_2, and putting a dot of enamel paint (Humbrol Ltd, UK) on their thorax, but the flies then flew too far away and very few came back to hover at the studied spot. The same procedure without the anaesthesis was no more successful. The polymorphism associated with abdominal coloration helped in identifying flies over the half hour recording periods, but accurate identification over a single day and indeed the whole experiment was impossible. As a consequence, the records are certainly not independent: several records were obtained for single flies. Thus, the assumption of independence of the data for statistical analysis is not met. An analysis was carried out on the whole data set, bearing this problem in mind. It allowed me to suggest which factors affect hovering duration. Time of day, ambient temperature, light levels and relative humidity were considered. The difficulty with such climatic data is that they are all correlated to some extent. When too many correlated variables are included in a regression analysis these factors become non significant predictors of the model. It was decided to eliminate the humidity factor as the one least likely to have an influence. *E. pertinax* is probably not water stressed (see Chapter 3), and humidity, in the range observed during this study, is certainly not the factor which influences most hovering. Moreover, hovering males were seen several times extruding water from their anus, suggesting they have a water surplus. So, the pooled data were analysed for the influence of the time of day, ambient temperature and light. The time of day is obviously an important factor; the flies are not active at night. This could be because it is dark, but darkness is probably only part of the explanation as many insects have intense circadian activity patterns (e.g. Helfrichforster et al 1998, Lewis et al 1991, Meyerpeters 1993, Moore et al 1998, Sothern et al 1998). It is often left out of these kinds of analysis as the other climatic factors are well correlated with it and hygrothermal constraints are

thought to be more important. However on overcast days, light and temperature might remain fairly constant throughout the day, but some insects might still show a peak of activity. Moreover, conditions of light and temperature differ every day, but insects might still peak at a similar time. Time of day is not influencing flies' activity in a linear fashion, so a quadratic model has to be fitted. As it could not be ascertained if the Minitab statistical package could handle both linear and non-linear model fitting in one operation, first the quadratic model was fitted and then a linear multiple regression (with temperature and light as factors) on the residuals was carried out. Thus, the influence of temperature and light on the flies' activity could be investigated with time of day being controlled for.

To account for the lack of independence of the data, and to support the positive or negative relationship between two variables suggested by these tests, it could be shown that above an arbitrary point, the data points do not belong to the same population as those below that point (Mike Ritchie pers. comm.). For example, the influence of temperature on the duration of hovering was investigated and the regression analysis suggested that a positive relationship exists between the two variables. To confirm this, it was shown that the data for hovering duration above an ambient temperature of 19 °C did not belong to the top 5 % of the distribution for the data below 19 °C. Thus above 19 °C, hovering duration is higher than below 19 °C, and there is a positive relationship between temperature and hovering duration.

The suggestions made by the analysis of the whole data set were confirmed by looking in more detail at two days contrasting in their climate. One was a cool, bright day (3.6.97), the other a warm, bright day (10.6.97). Light levels were similar, so this influence could be eliminated. Time of day could be ignored as hovering duration was compared at a similar time. Thus, the influence of temperature on hovering duration could be investigated. Its significance was obvious from the graphs, but was also shown using regressions and the method outlined above.

7.2.2 Flies' abundance at foraging sites

The abundance of the two eristalines coming to forage was recorded at one main site, a patch of oregano (*Origanum marjorana*), a small labiate, at Dundee Botanic Garden (map reference NO 368 286) in summer 1997 (Figure 7.2a). One day of observation was also carried out at St Andrews Botanic Garden (map reference NO 502 163) on a patch of aster, *Aster pyranacus*, a composite (Figure 7.2b). An area of 1.2 x 1.6 m (1.92 m^2) was delimited on the flower-bed using green threads. Observations were done from about 09.00 h until no fly was on the patch (usually around 18.00 - 19.00 h, except on 20.8.97 when it rained at 16.00h). Four full days of observations were obtained for the oregano patch (8, 14, 20 and 25 August 1997).

Oregano presents small flowers in group of about 6, these groups are themselves grouped in "flower heads" along the upper part of the stem. There were 43 ± 5 plants in an area 0.25 x 0.25 m (0.0625 m^2). The average number of flowers offered by each plant was 100 ± 26. Many flowers had already fallen from the plant. Therefore, the studied area offered around 132000 ± 15000 (43 ± 5 x 100 x 30.72 (area = 30.72 x 0.0625 = 1.92 m^2)) flowers on each day of the study.

Air temperature in sunshine and shade, and temperature at the level of the flowers, were recorded every 10 min by a four channel data logger (Squirrel meter/logger, Eltek Ltd, UK). This data logger also recorded light levels in full sunshine. Humidity was measured every half hour in the sunshine about 1.50 m above the ground and just above the flowers with the hand held humidity meter HMI 31 probe. Wind speed was obtained every half hour (2 m above the ground) with the hand held anemometer, but these data were not used in the subsequent analysis as at the speeds measured foraging was not influenced by wind.

Nectar was collected with 1 µl capillary tubes (Drummond Scientific Co., USA). Between 20 and over a hundred flowers were sampled each half hour (depending on the number of empty flowers). The volume was calculated from the length of the column measured with callipers to the nearest hundredth of millimetre, and was expressed as volume per flower. Concentration was determined using a modified pocket refractometer (Bellingham and Stanley, Ltd, UK) which accepts minimum volumes of 0.05µl. Attempts were made to measure nectar availability on aster by

a/

b/

Fig. 7.2 Flower-beds for the study of foraging

a/ Oregano, Dundee Botanic Garden
b/ Aster, St Andrews Botanic Garden

using drawn out pipettes, but the difficulty associated with collecting any nectar provoked the abandonment of the idea. No attempt was made to collect pollen.

Flies' numbers were recorded for 10 minutes every half hour. Each fly entering the delimited area was counted. Thus, some flies which left the area and later returned were counted twice or more. The sex of the flies was determined. It was also noted if the flies were taking nectar, pollen or both.

Four factors were considered here: temperature (air in sunshine, air in shade, or just above the flower bed), light levels, humidity and time of day. After some preliminary analyses, it was decided to eliminate the humidity factor. Humidity is highly correlated with temperature, and of the two factors, temperature is more likely to influence the flies' abundance. Early in the morning, a low temperature is probably going to be a more important constraint to activity than a high humidity. During the hottest part of the day both temperature and the low humidity could be stressing the flies. However, on the hottest day of this study (14 August 1997), relative humidity did not get much lower than 50%. Thus, in the climatic conditions of these four days, temperature is a more probable constraint than humidity on flies. In addition, *E. pertinax* abundance correlation with climatic factors was the worst with humidity ($r = -0.113$). Of the three temperatures that were measured, the temperature just above the flower bed was found to have the most influence on the flies' foraging. When the analysis of these data was first started, time of day was left out. However, in view of the low percentage of the variation of flies' abundance explained by the model, it was subsequently included.

7.2.3 Flower handling time

Flower handling time was measured for oregano only, because data about nectar availability could only be obtained for this plant. The time spent on the patch by a fly was recorded using a stop watch and the number of flowers sampled counted. This time included time spent to walk from flower to flower. If two successive flowers were too distant and the fly stopped feeding for a considerable time, recording was discontinued. These data only concern nectar collecting. One full day of observations was done and one record was also obtained for another day

(overcast day). Nectar profiles were not measured for this study. Instead, records from the foraging study were used as an indication of nectar availability.

7.3 Results

7.3.1 Hovering

A/ Field observations

a/ *E. tenax*

As already mentioned, hovering in male *E. tenax* was very rarely observed in Scotland. Very few such records were made over the three years of this project. The first one was on 7 June 1997 at Kippford, a coastal village on the Solway coast (Dumfries and Galloway). Temperature was around 18- 20 °C (no measuring apparatus available), there were sunny spells, and the time was between 15.00 and 16.00 h. Two males were seen hovering along a hedge. They hovered at about 0.5 m above the ground, only when the sun was out and for short periods only (less than a minute). There was no wind. Several males and females were observed feeding on *Cotoneaster* sp. nearby.

The second record was made on 19 July of the same year in Dundee Botanic Garden, where a male *E. tenax* was seen hovering again along a well-sheltered hedge in full sunshine and about 0.5 m above the ground at 14.00 h; the ambient temperature was 26.5 °C.

The third record was on 21 July 1997 at St Andrews Botanic Garden, where a male was hovering along a beech hedge. Ambient temperature was 19.2 °C

Disturbance meant that the males flew away and did not come back for several minutes.

b/ *E. pertinax*

Figure 7.1 shows a hovering male *E. pertinax*. Hovering in male *E. pertinax* is quite in contrast with that of *E. tenax*. Males are very often observed hovering, usually near shrubs in sheltered places but in sunshine. They were for example seen in an alley bordered by tall shrubs

and trees at St Andrews Botanic Garden, along the edge of a deciduous forest (in France) and on a path bordered by gorse in the countryside near Perth (Perthshire). They are quite conspicuous and hover between 1 m to 2.5 m from the ground. This hovering behaviour is very common from the end of May to the end of July. Interestingly, only one male was sighted hovering in the autumn: on 5 September 1997 in St Mary's Quad, St Andrews. Again the fly was near a hedge; ambient temperature was 19.2°C. Hovering behaviour in *E. pertinax* therefore seems largely limited to late spring-early summer.

An anecdotal observation was made on 19 September 1997. A male *E. pertinax* was resting on a bush's leaf, in the sunshine, along one of the paths at St Andrews Botanic Garden. When very small stones were thrown in the air near the bush, the fly darted to them. After each chasing, it came back to the same leaf. Was this fly trying to intercept passing females? This would suggest a change in reproductive strategy from territory defending by hovering in early summer to a "perch-and-pursue" strategy in autumn. It would certainly be worth investigating this further.

The hovering behaviour being common, a detailed study could be undertaken. Hovering alternates with periods of rest and/or basking. It was difficult to be certain if the "resting" periods were purely resting or if they were associated with basking. However, it was observed that most of the time, *E. pertinax* rested on leaves that were oriented towards the sun. Their body was thus also oriented towards the sun, and very often the wings were held apart, uncovering the abdomen. This suggests that basking is associated, at least part of the time, with these resting periods.

Numerous short hovering bouts (of a few seconds) were observed (with a resting period in between) at all temperatures and times of the day at which observations were made. However, amongst these short hovering bouts some longer ones were recorded. The longest recorded was 558 seconds (9 min 18 s). Therefore, during the period a single fly was observed, there were always short hovering bouts lasting from 1 to 60 seconds, and occasionally longer bouts.

While hovering, *E. pertinax* did not seem disturbed by the presence of an observer. They very often hovered right overhead. When an attempt to catch one of the flies with a hand net failed, the fly was back hovering at the same position within a few seconds. *E. pertinax* hover in one position,

moving their head from side to side, as if scanning their surroundings. They periodically make a sharp turn on the same spot, presumably to watch another area. When another insect passes by, the hovering *E. pertinax* darts to that insect and comes back to hover at the same spot as before if it is not a female of its own species. Sometimes, it flies away in tandem with another insect, supposedly a female of its species in order to copulate, although that could not be verified. There were flowers near the hovering spot, but male *E. pertinax* were not seen to interact with females at the flowers. As flies could not be marked, it is not certain if a male that had flown away with a female came back. Certainly, the hovering spot was usually occupied soon after, but maybe not by the same male. Quite often, males hover close to each other and to other hovering syrphids, such as *Volucella pellucens*, occasionally chasing each other. Also, it could not be ascertained that an individual comes back to the same site on successive days. However, when a fly was captured and removed permanently to measure its thoracic temperature, very soon after another male was seen hovering at the same spot. Perhaps some males take the opportunity to move to a better spot than they had before. There were certainly many males around ready to take up the place of the captured one. These observations, although very preliminary, suggest that there might be some intra- and inter- specific competition for a suitable territory to defend.

B/ Variations in hovering duration - the influence of the climate

Observations were carried out on six days, end of May - beginning of June 1997. The present section examines how hovering duration varies during the day. The pooled data for the six days were analysed to investigate the overall effect of climate on hovering duration. Time of day (t) is a significant predictor of maximum hovering duration but not of mean hovering duration (mean duration: $n = 81$, $R^2 = 0.074$, $t\ p = 0.122$, $t^2\ p = 0.092$; mean maximum duration: $n = 81$, $R^2 = 0.113$, $t\ p = 0.010$, $t^2\ p = 0.007$). Once time has been controlled for, the analysis suggests that ambient temperature has a strong effect on hovering duration whereas light does not (mean duration: $n = 81$, $R^2 = 0.06$, $T_a\ p = 0.032$, $L\ p = 0.900$; mean maximum duration: $n = 81$, $R^2 = 0.100$, $T_a\ p = 0.005$, $L\ p = 0.961$). The mean of the data for temperatures above 19 °C (once time had been

controlled for) did not belong to the 95 % confidence interval of the data for temperatures below 19 °C, supporting the influence of temperature.

Hovering on two climatically contrasting days is described below. The 3rd of June 1997, was a bright, cool day. Ambient temperature did not get higher than 18.1 °C and was 15.3 °C at 09.45 h and 15.8 °C at 16.30 h (Figure 7.3a & b). In contrast on 10 June, ambient temperature was 19.5 °C at 10.00 h, peaked to 22.3 °C at 13.00 h and decreased down to 17.8 °C at 17.00 h (Figure 7.3c & d). Thus, temperature conditions were quite different. However light conditions were more similar (Figure 7.3b & d). *E. pertinax* rarely hovers on overcast days. Perhaps it would do so if ambient temperature was high enough, but this could not be checked as such a warm cloudy day never materialised during the study period. Figures 7.3b & d show the daily pattern of temperature, light and relative humidity. Light and temperature usually increase in the morning, peak around midday and decrease in the afternoon. Relative humidity tends to do the opposite, although the presented data show much variation. As already mentioned, humidity was omitted from this analysis. In addition, it was shown above that overall, light has no influence on hovering duration once time and temperature are controlled for. Therefore, temperature is the climatic factor that is going to be considered here as representing the climate.

Figures 7.3a & c show that on both days, there was usually no hovering before 10.00 h (only once during the six days was a male seen to hover before that, at 09.15 h) or after 17.00 h. In between these times, there were always some males hovering, but the mean and maximum duration of their hovering also changed with the course of the day. Duration peaked in early afternoon. Comparing Figure 7.3a and Figure 7.3c , it is clear that these peaks of hovering duration correspond to temperature peaks. Moreover, hovering duration was higher on the warmer day (10.6.97) than on the cooler day (3.6.97). The maximum duration (9 min 18 s) was recorded on 10 June at 13.00 h at 22.3 °C. In contrast, the maximum duration recorded on 3 June was 2 min 49 s at 14.00 h at 18.1 °C.

The effect of temperature was further analysed by regression analysis of hovering duration (mean and maximum) on ambient temperature. There is a significant linear relationship between maximum

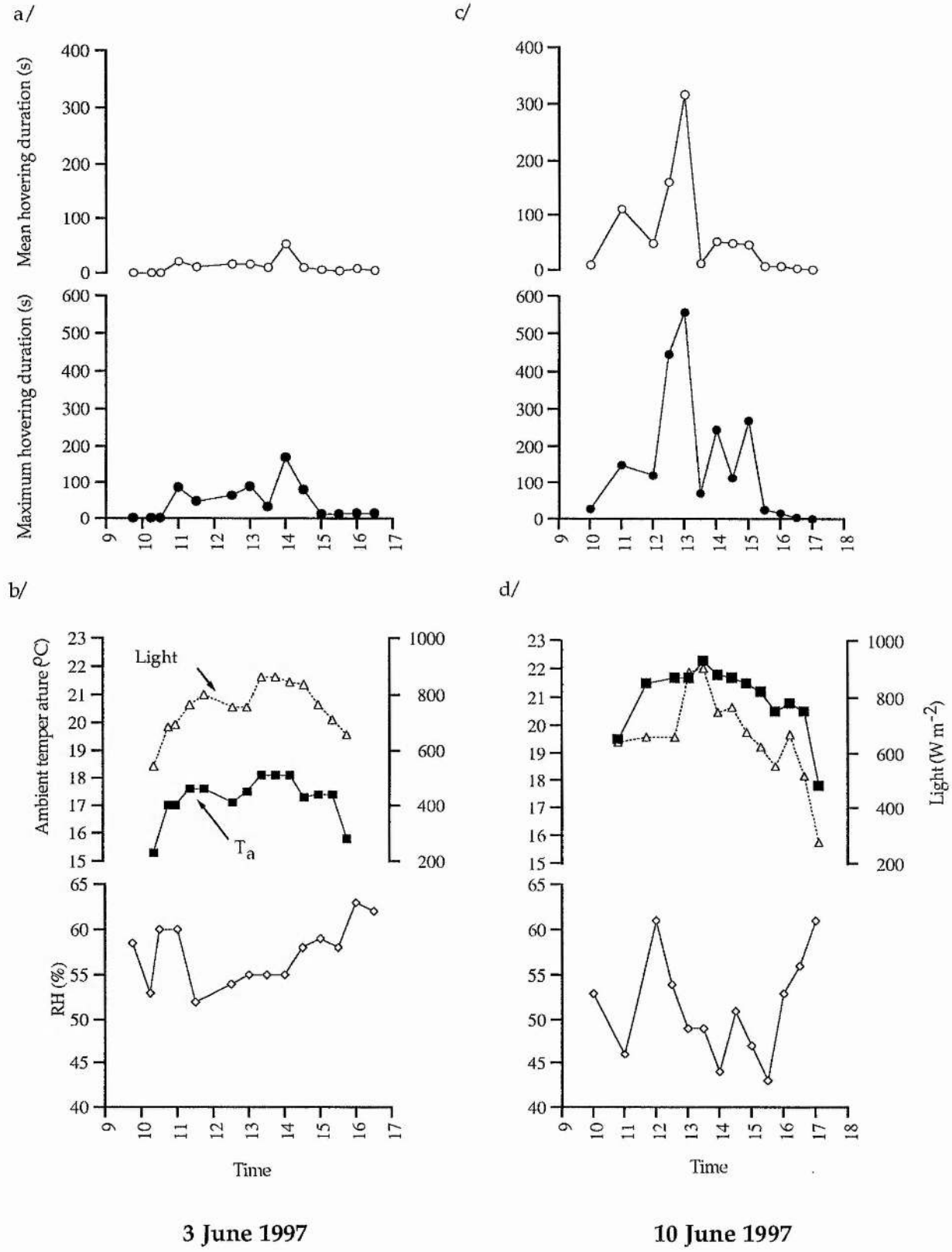


Fig 7.3 Daily variation of climatic factors and hovering duration in male *E. pertinax* on a cool day (a and b) and on a warm day (c and d)

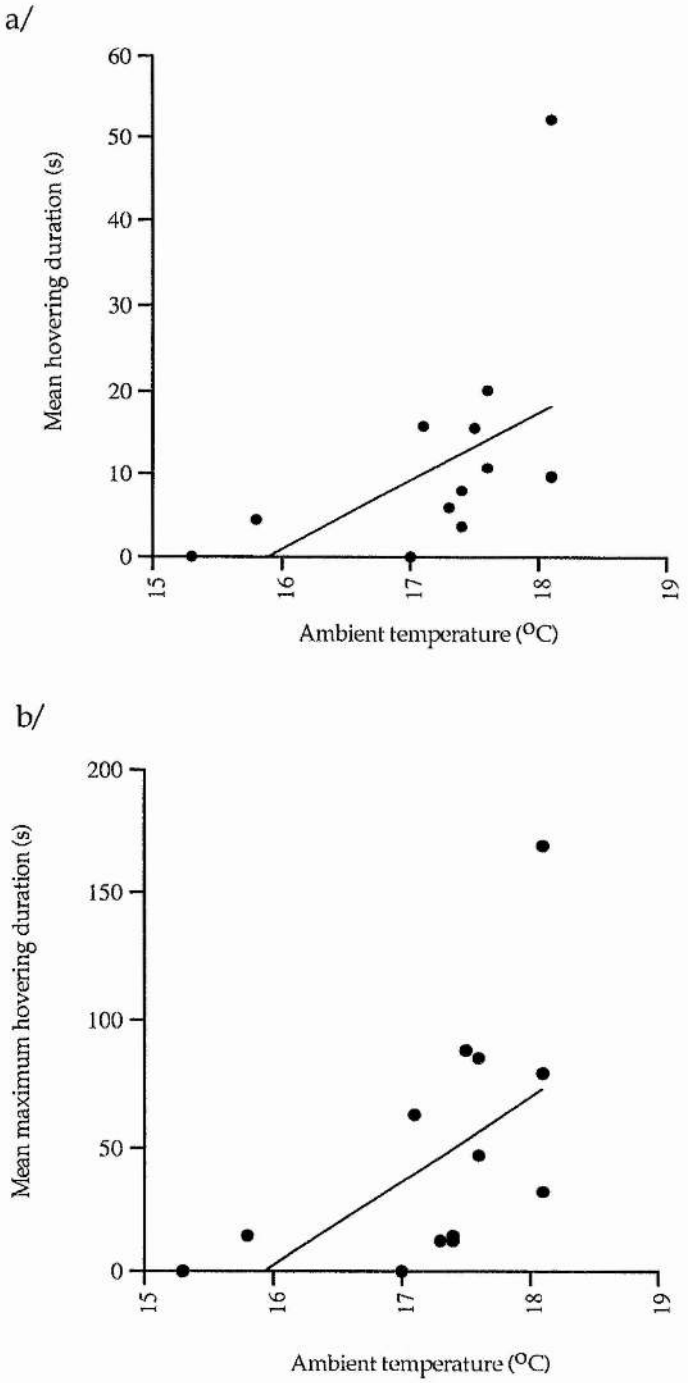


Fig 7.4 Influence of temperature on hovering duration on 3 June 1997 (cool day)

a/ Mean hovering duration vs temperature, $y = 8.284x - 131.659$ $r^2 = 0.253$
b/ Mean maximum duration vs temperature, $y = 33.800x - 538.633$ $r^2 = 0.320$

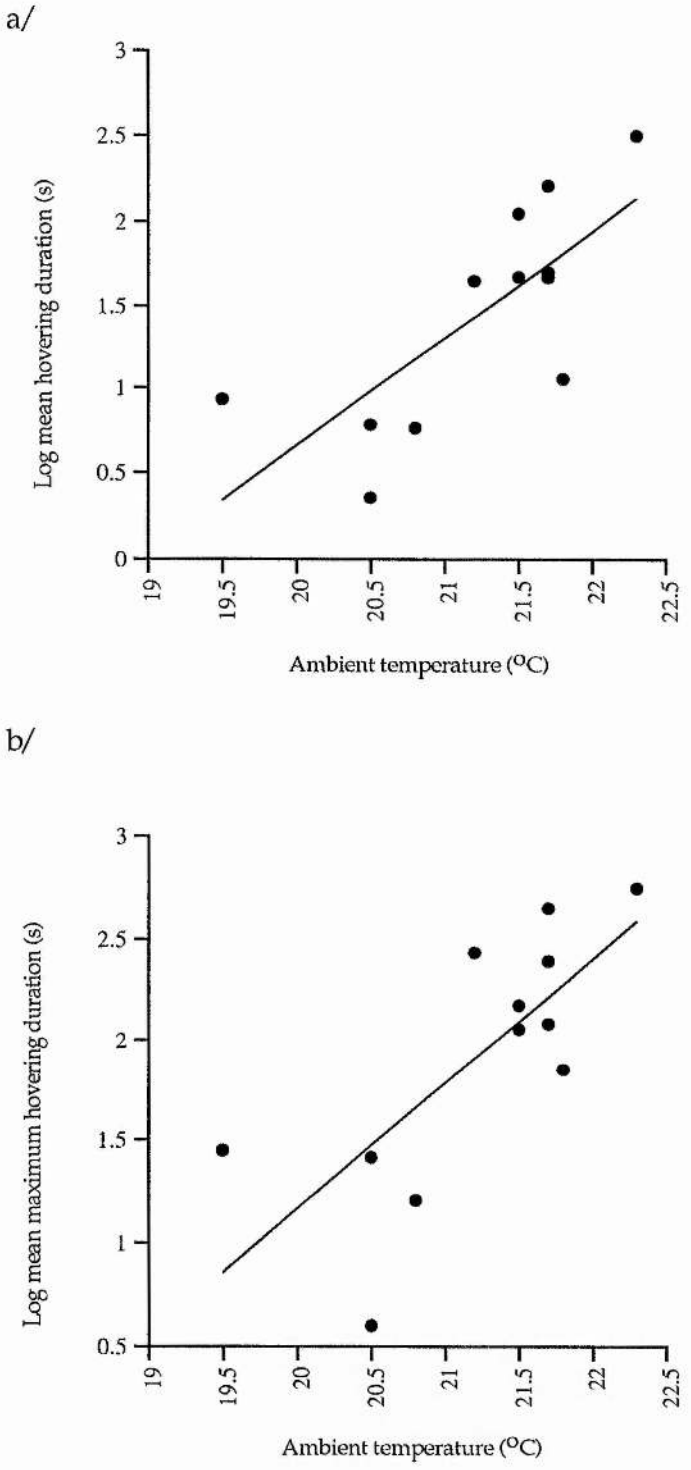


Fig. 7.5 Influence of temperature on hovering duration on 10 June 1997 (warm day)

a/ Mean hovering duration vs temperature, $y = 0.641x - 12.168$ $r^2 = 0.559$
b/ Mean maximum duration vs temperature, $y = 0.619x - 11.206$ $r^2 = 0.549$

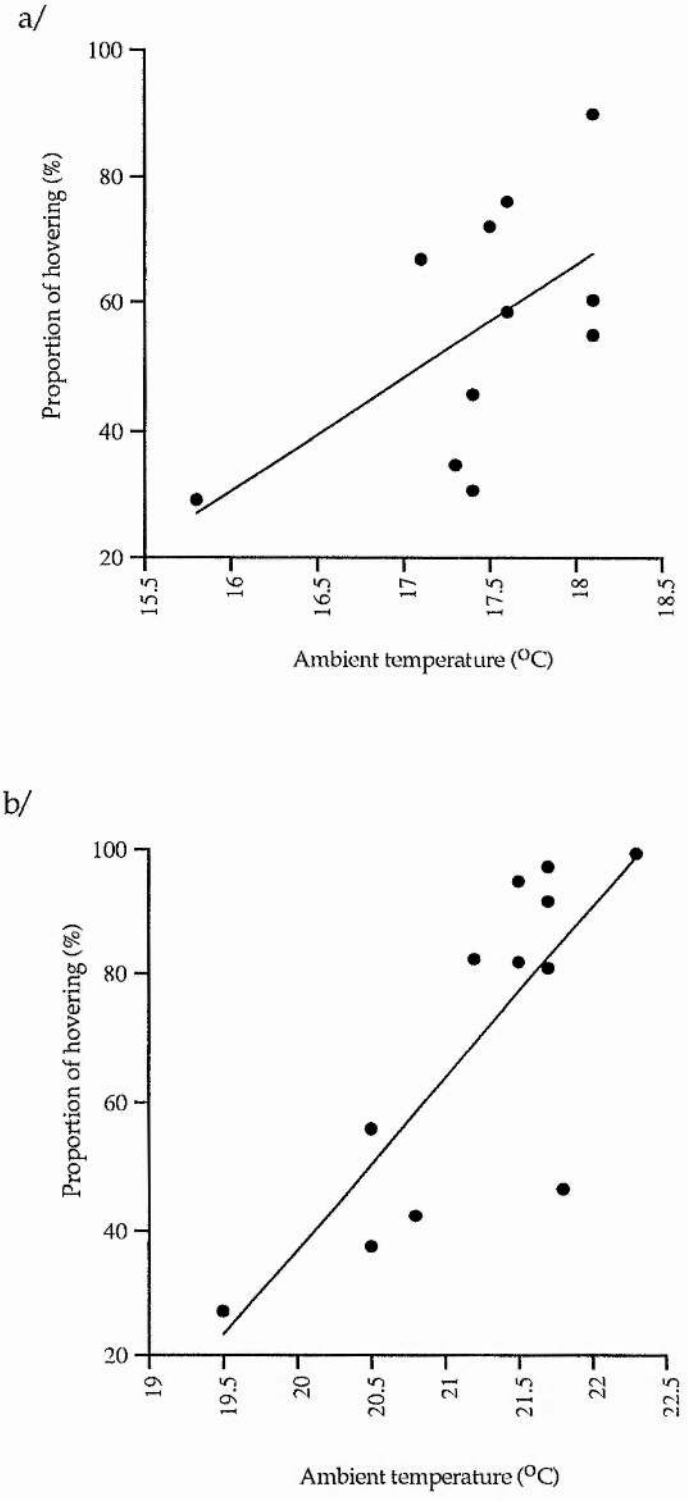


Fig. 7.6 Influence of temperature on the proportion of hovering relative to resting

a/ 3.6.97 (coold day), $y = 17.590x - 250.741$ $r^2 = 0.336$
b/ 10.6.97 (warm day), $y = 27.011x - 503.461$ $r^2 = 0.631$

hovering duration and temperature (n = 14, R^2 = 0.320, p = 0.035) (Figure 7.4b) and an almost significant linear relationship between mean hovering duration and temperature (n = 14, R^2 = 0.253, p = 0.067) (Figure 7.4a) for the cool day. The mean of the data for temperatures above 17.5 °C does not belong to the 95 % confidence interval of the data below that temperature. For the warm day, both maximum and mean hovering duration have a logarithmic relationship with temperature (maximum duration: n =13, R^2 = 0.593, p = 0.003; mean duration: n = 13, R^2 = 0.581, p = 0.004) (Figure 7.5a & b). The mean of the data for temperatures above 21.5 °C does not belong to the 95 % confidence interval of the data below that temperature.

The proportion of time spent hovering (as the fraction of the total time, made up of hovering and resting) was also analysed in relation to temperature. Data where no hovering was observed (that is before 10.00 h and after 17.00 h) were not used here, as the flies were not seen so their activity is unknown. When the proportion of hovering is plotted against temperature (Figure 7.6) a highly significant positive relationship is found on 10 June (n = 12, R^2 = 0.631, p = 0.002) and an almost significant positive correlation exists for data from 3 June (n = 11, R^2 = 0.336, p = 0.061). These results suggest that as temperature increases, male *E. pertinax* spend more time hovering than resting/basking. Again, these relationships were confirmed by checking that the mean of the data above a certain temperature did not belong to the 95 % confidence interval of the data below that temperature.

7.3.2 *E. tenax* and *E. pertinax* foraging

Preliminary studies in summer 1996 showed that these two eristalines forage frequently on small labiates such as mint and oregano and on composites such as ragwort and asters (Fig 7.7). This study aimed to investigate the effect of climate on the flies' abundance and also looked at the nectar reward they get while foraging. This study was carried out on oregano which is a much more suitable plant for such investigations than composites. Nevertheless, some observations were carried out on syrphids foraging on aster and some reference will be made to them.

a/

Fig. 7.7 Eristalines feeding
a/ Female *E. tenax* on ragwort

b/

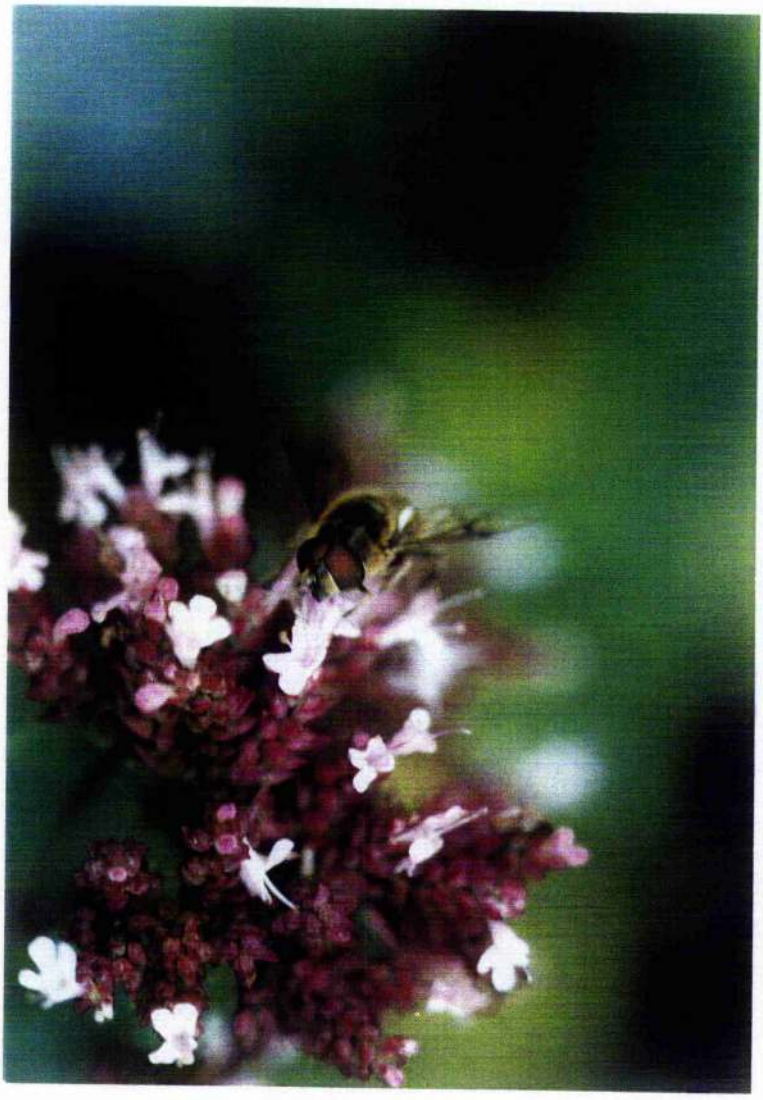


Fig. 7.7 Eristalines feeding
b/ Male *E. pertinax* on oregano

c/

d/

Fig. 7.7 Eristalines feeding
c/ *E. tenax* collecting nectar on oregano
d/ Male *E. tenax* on oregano

e/

f/

Fig. 7.7 Eristalines feeding
e/ Female *E. tenax* on aster
f/ Female *E. pertinax* on mint

Both species rarely took pollen from oregano flowers; they forage essentially for nectar. In contrast, both pollen and nectar were frequently collected from aster flowers.

Figures 7.8a, b, c & d show the abundance of these two species, climatic factors and nectar volume and concentration during each day during August 1997. The influence of climate on foraging was analysed on the data for the four days pooled because independence of data could be assumed and (in contrast to the hovering data) there were not very striking climatic differences over this period. However, some reference will be made to particular aspects of single days. In addition, the influence of temperature on the flies' abundance on 14 August 1997 will be discussed separately because this was the hottest day of the study, and thus the flies were particularly likely to experience thermal stress.

A/ Daily variation in flies' abundance

Figure 7.8 shows how the flies' frequency, nectar concentration and volume and the climatic factors varied throughout each day of the study, and Figure 7.9 represents the data of the four days pooled together. From these figures, it is clear that the flies' abundance varies throughout the day and tends to peak around midday, more or less at the same time as temperature and light. It is also clear that males of both species tend to be more abundant than females, and that *E. tenax* predominates (except on 14 August, the hottest and brightest day). However, observations done on aster on 19 September show that females dominated males for *E. pertinax* and occurred in similar numbers to males for *E. tenax*. On this plant, *E. pertinax* were more numerous than *E. tenax*, but this was due to the increase in females' numbers (Figure 7.10).

In oregano, the volume of nectar is high in the morning and decreases throughout the day (Figure 7.8). More flowers contained nectar in the morning, but as the day advanced it became more and more difficult to collect any nectar. On 14 August, the amount of nectar available was higher than on any other day. This nectar was very dilute. This uncharacteristic food availability can easily be explained by the fact that it had rained heavily on the preceding night and the flower patch was very wet. In general, the maximum nectar volume available per flower (in

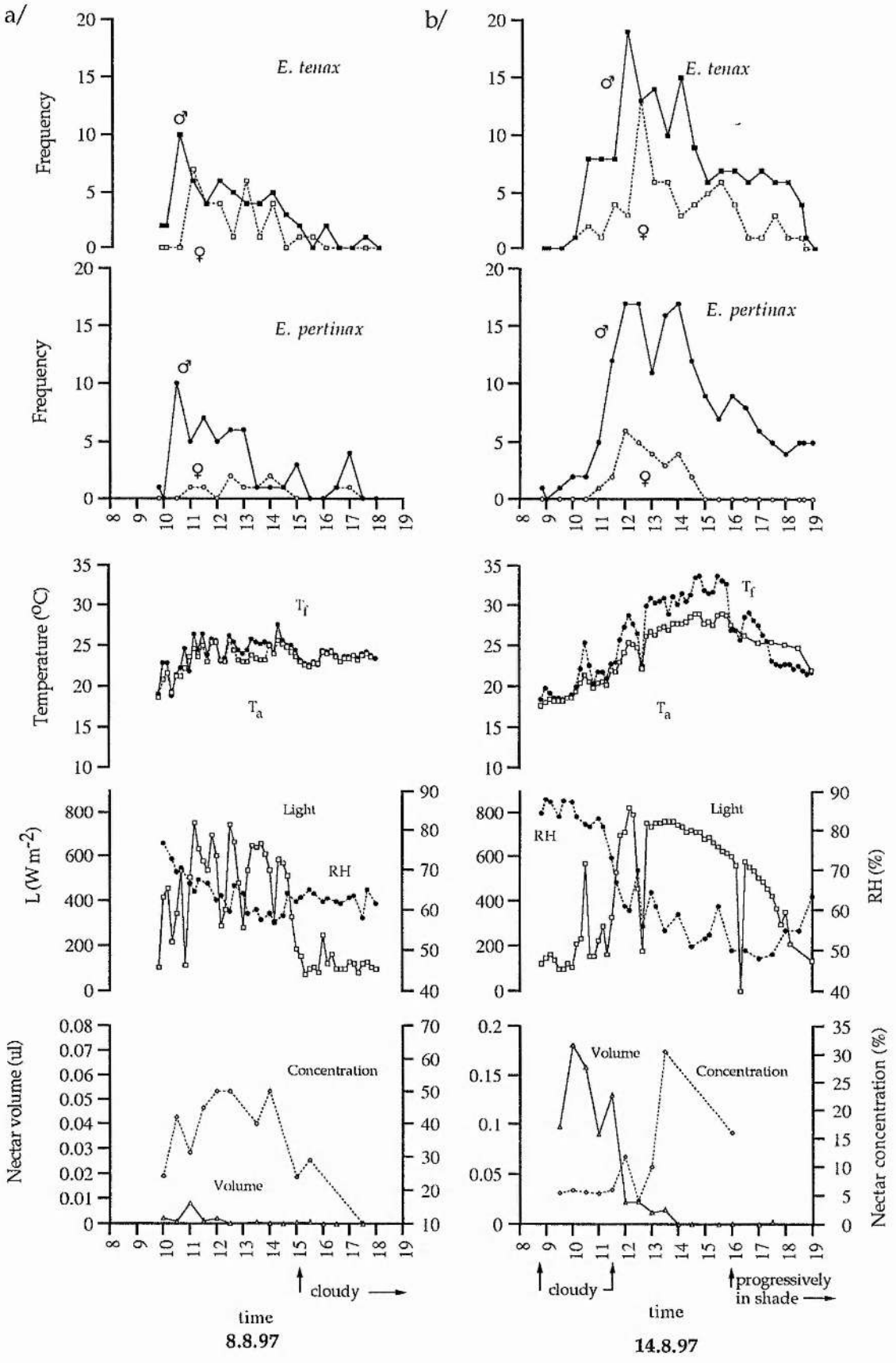


Fig. 7.8 Daily variation of flies' abundance, climate and nectar volume and concentration
NB Different scale for Y-axes for nectar data on 14.8.97

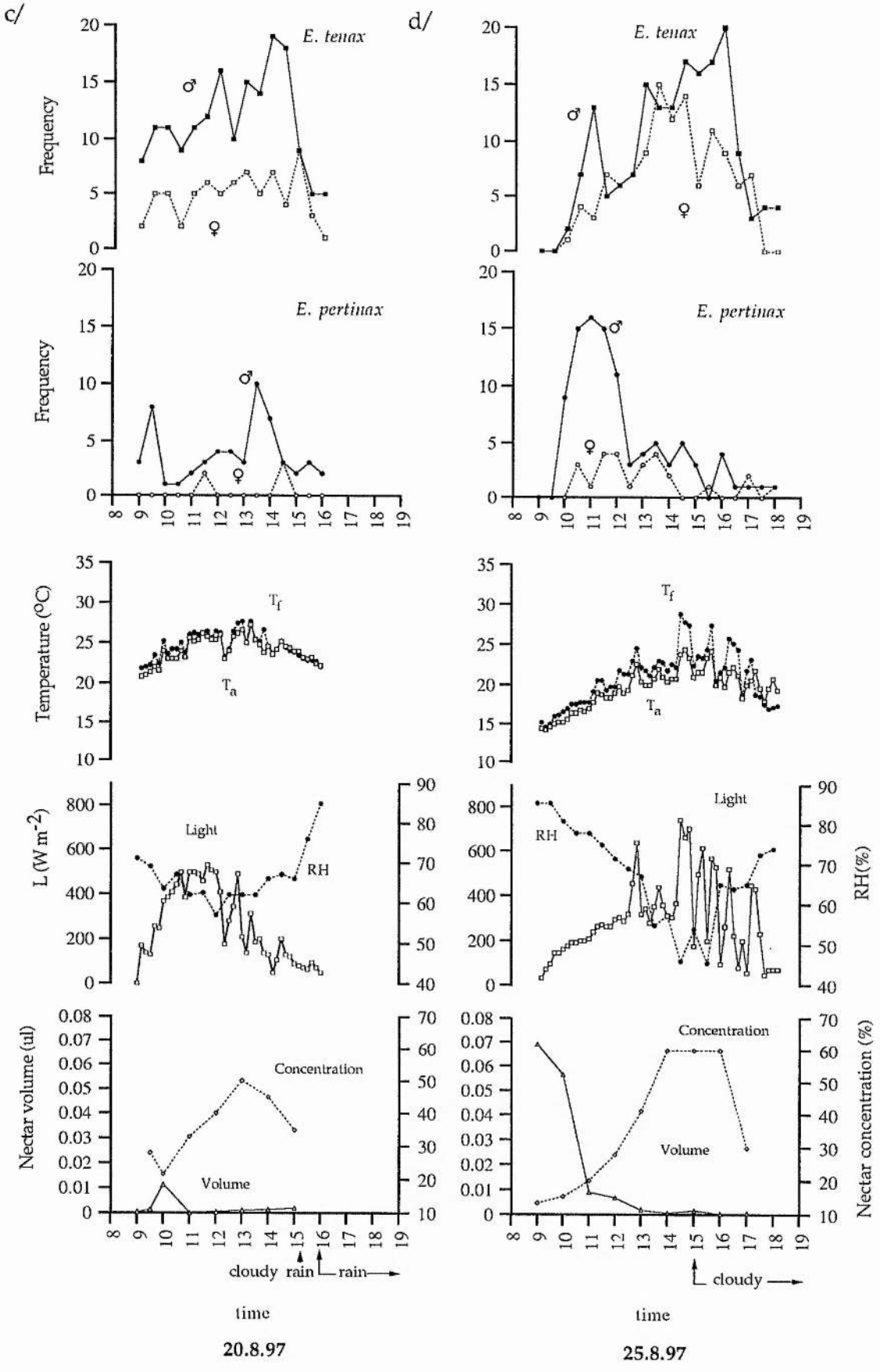


Fig. 7.8 Daily variation of flies' abundance, climate and nectar volume and concentration

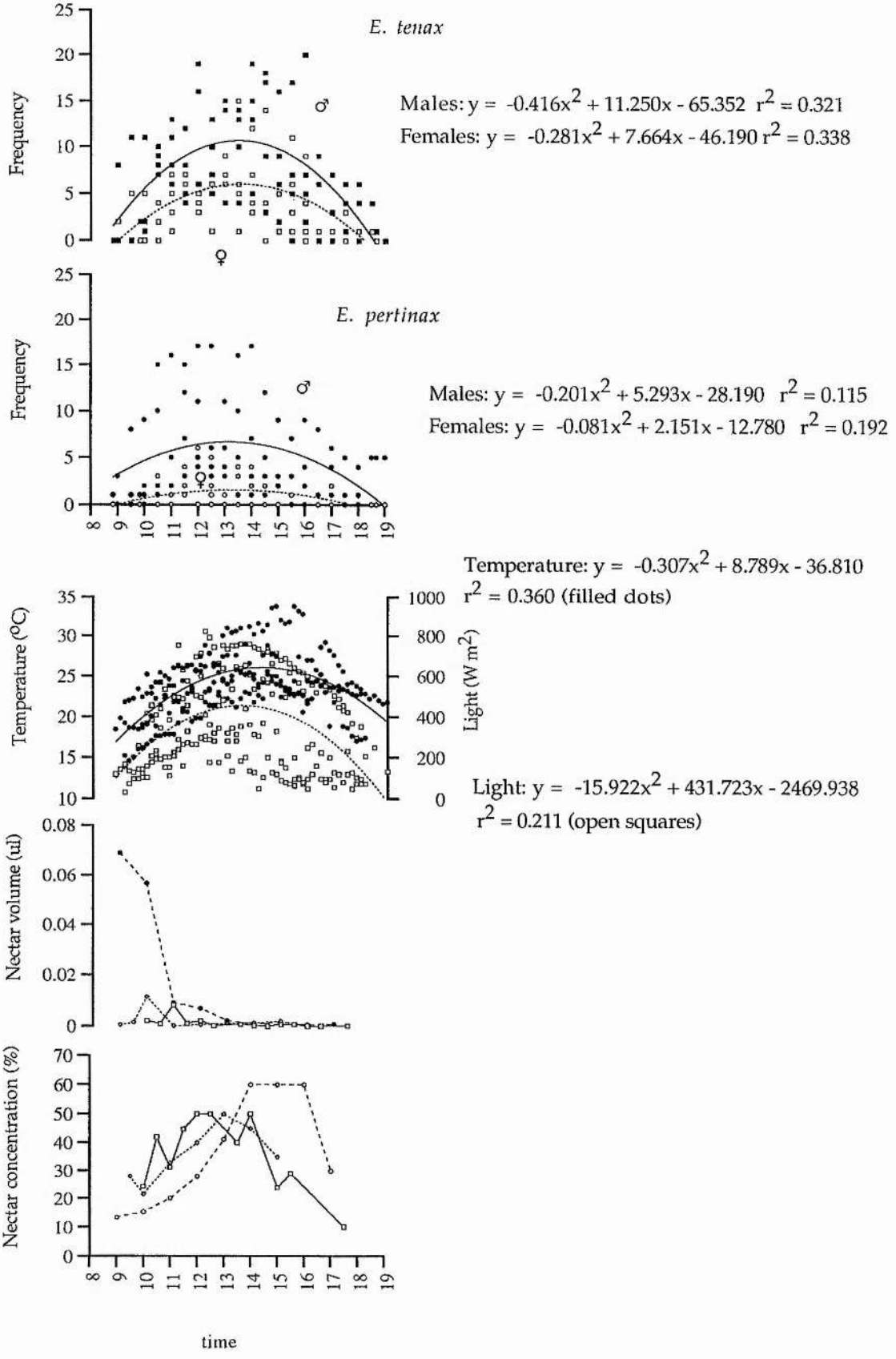


Fig. 7.9 Overall daily variation in flies' abundance, temperature at flower-bed, light, nectar availability and nectar concentration.
NB: Data for nectar not pooled; only 3 days shown

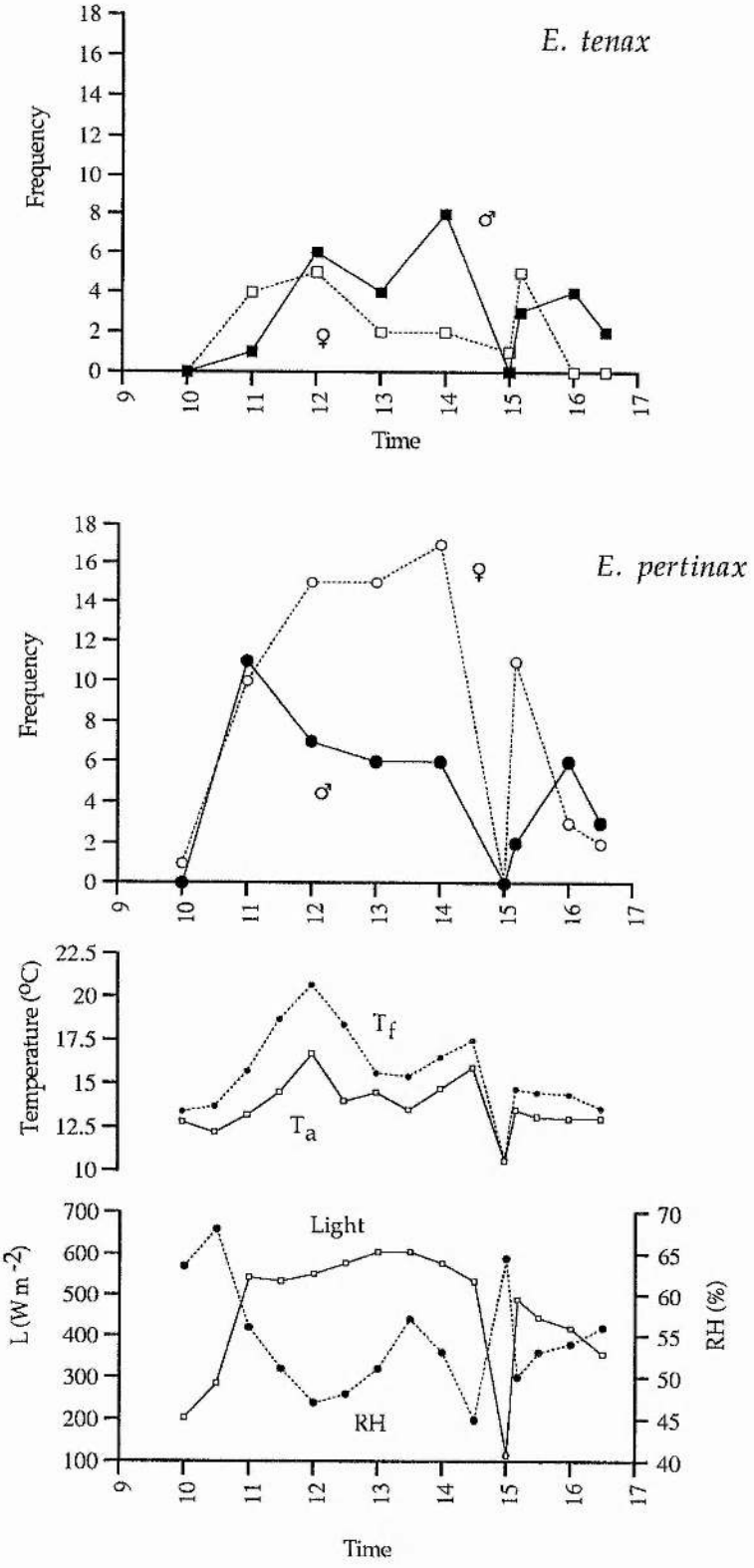


Fig. 7.10 Variation in flies' abundance, temperature, light and humidity on aster on 19.09.87

the morning) was between 0.008 and 0.07 µl. Even when the experimenter could not collect any nectar, the flower bed was buzzing with activity. It is obvious that bees and flies were able to collect food, and that the flowers still contained nectar which could not be sampled using capillary tubes.

Nectar concentration tends to be low in the morning and late afternoon and high around midday. It varies between 15 and 60% during the day and reflects closely the variation of humidity. Thus at the time they are most abundant, these eristalines find small volume of nectar per flower, at a concentration of 40-60%. It was not attempted to analyse the effect of nectar availability and concentration on the flies' abundance because they are interdependent. Volume decreases and concentration increases because of water evaporation, itself depending on weather conditions. The flies might prefer foraging when nectar is highly concentrated (higher energy reward per volume which has then to be carried) but their own foraging activities and those of other insects also cause nectar to disappear. Moreover, the flies' peak of foraging might also be influenced by the same climatic factors that provoke changes in nectar. It is very difficult to tease the effects of these various factors apart, but given that flies were just as numerous on 14 August, when nectar availability and concentration were strikingly different from those of the other three days, it seems more likely that climatic factors rather than nectar dictate the flies' foraging pattern.

The factors affecting the flies' abundance are analysed below in more detail for each species.

B/ *E. tenax*

Both males and females' abundance are strongly influenced by the time of day in a quadratic fashion (males: $n = 75$; $R^2 = 0.321$, t $p < 0.001$, t^2 $p<0.001$; females: $n = 75$, $R^2 = 0.338$, t $p < 0.001$, t^2 $p < 0.001$) i.e. *E. tenax* peak in abundance at around 14.00 h (Figure 7.9). However, light levels and temperature are apparently not significant factors once time is controlled for (males: $n = 72$, $R^2 = 0.017$, L $p = 0.667$, T $p = 0.321$; females: $n = 72$, $R^2 = 0.006$, L $p = 0.539$, T $p = 0.713$). Thus the time of day seems really to be the cue they use. These results would suggest that the time of day has a strong influence on *E. tenax* foraging, but that temperature and

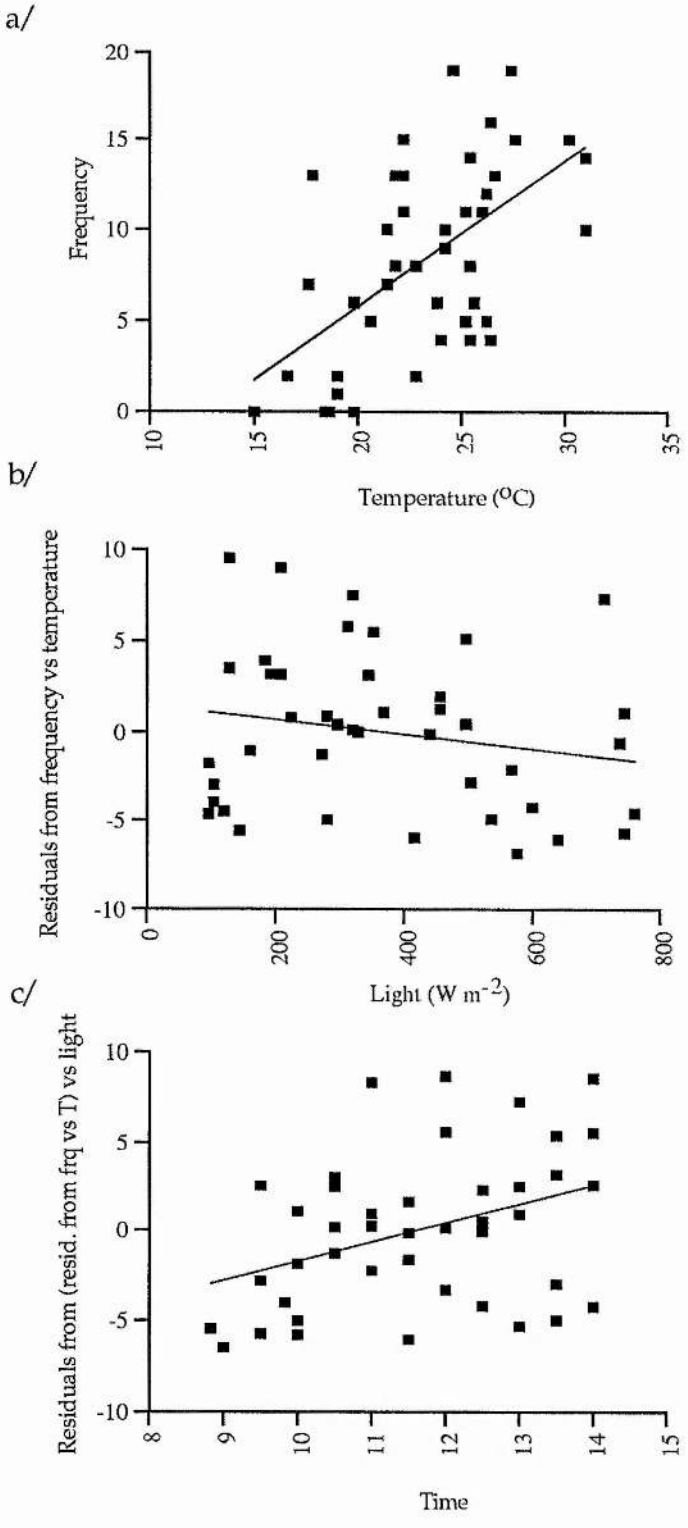


Fig. 7.11 Influence of climate on male *E. tenax* abundance before 14.00 h

a/ Flies number versus temperature at the flower bed, y = 0.802x - 10.287 $r^2 = 0.324$

b/ Residuals of a/ versus light, y = -0.004x + 1.478 $r^2 = 0.035$

c/ Residuals of b/ versus time, y = 1.074x - 12.478 $r^2 = 0.145$

light levels have none. However, when Figure 7.8 is analysed in detail, it is clear that the time at which *E. tenax* peaks varies from day to day and is very much related to the peak in temperature and light, with the exception of the hottest day of the study (14.8.97) which will be dealt with below. It is possible that the time of the day directs more the appearance and disappearance of flies, but that the peak in abundance is under the influence of other climatic factors. To test this idea, the same analysis as above was carried out on the data collected before 14.00 h, i.e. before the peak, when flies number increases. This time, a multiple regression on the flies' abundance for time, temperature and light could be done because the time factor has a linear influence on abundance (quadratic models were fitted to check this assumption and the time square factor was non significant in both cases). This analysis reveals that male *E. tenax* appearance is influenced by the time of day but also by temperature and to some extent by light ($n = 42$, $R^2 = 0.473$, t $p = 0.02$, T $p = 0.004$, L $p = 0.043$). The number of males increases with time and temperature (Figure 7.11a & c). However, light seems to have a weak negative relationship with fly number (Figure 7.11b). The appearance of females, on the other hand, seems to be influenced only by the time of day ($n = 42$, $R^2 = 0.417$, t $p < 0.001$ T $p = 0.549$, L $p = 0.140$).

C/ *E. pertinax*

Like *E. tenax*, *E. pertinax* respond to the time of day for foraging with a peak of abundance around midday as well (males: $n = 75$; $R^2 = 0.115$, t $p = 0.007$, t^2 $p = 0.005$; females: $n = 75$, $R^2 = 0.192$, t $p < 0.001$, t^2 $p < 0.001$). However, the variation in fly number accounted for by the time of day is much less than for *E. tenax*. *E. pertinax* respond to other climatic factors as well. Linear regressions on flies' number (controlled for the effect of time) for temperature and light reveal that males' and females' abundance is influenced by light levels; females' frequency also has a weak negative relationship with temperature (males: $n = 72$; $R^2 = 0.103$, L $p = 0.016$, T $p = 0.351$; females: $n = 72$, $R^2 = 0.084$, L $p = 0.015$, T p 0.048; Figure 7.12). Therefore, *E. pertinax* forage according to the time of the day and light levels. Possibly, temperature has a negative effect on females' abundance, but it is more likely that this is an artefact coming from the fact that temperature tends to peak after light (Figure 7.8). As females peak with

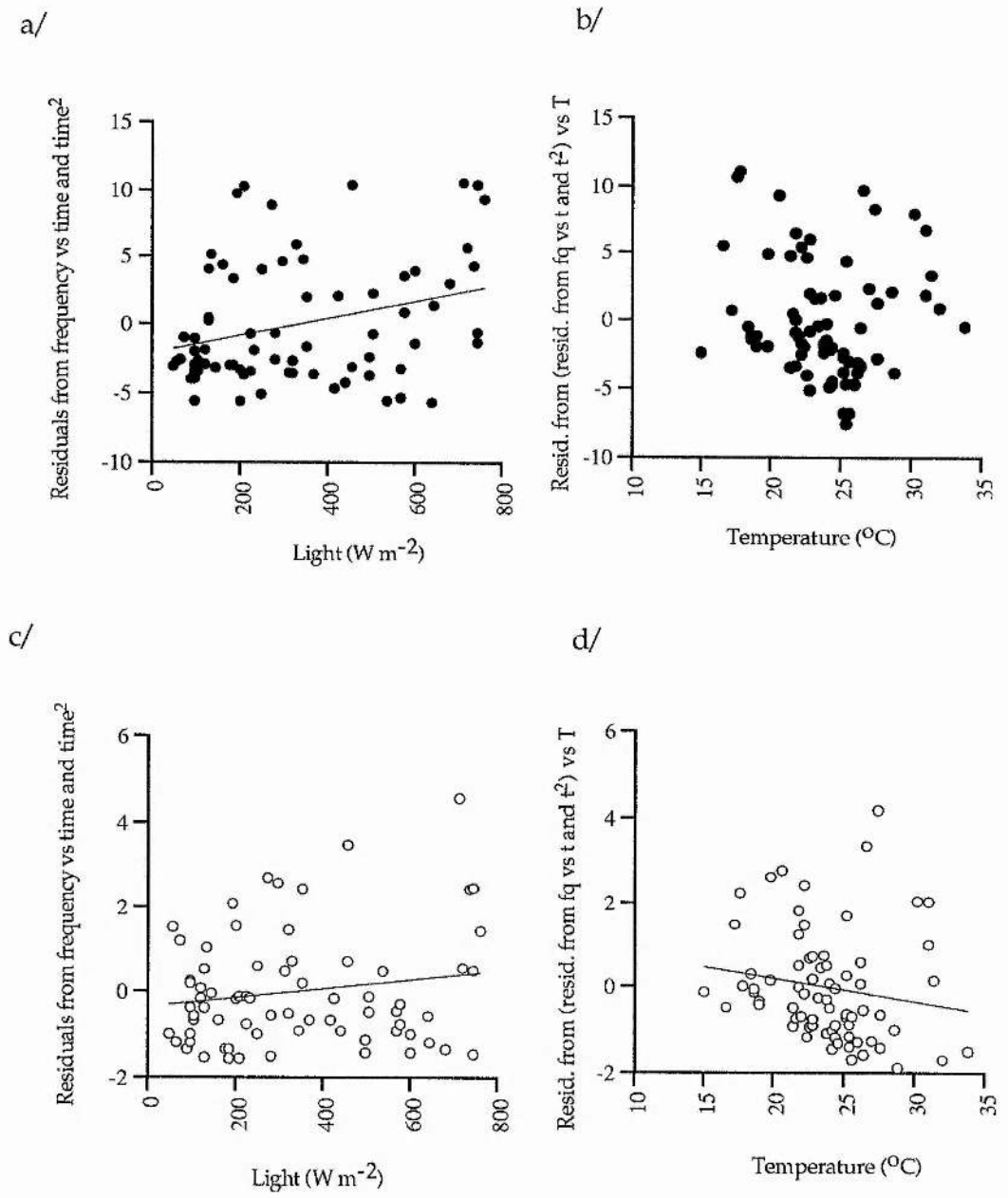


Fig. 7.12 Influence of light and temperature on the abundance of *E. pertinax* (time controlled for)

a/ Male *E. pertinax*: residuals from quadratic regression of frequency vs time against light, $y = 0.006x - 2.127$ $r^2 = 0.092$
b/ Male *E. pertinax*: residuals from a/ against temperature at flower bed
c/ Female *E. pertinax* residuals from quadratic regression of frequency vs time against light, $y = 0.001x - 0.358$ $r^2 = 0.030$
d/ Female *E. pertinax*: residuals from c/ against temperature at flower bed, $y = -0.054x + 1.285$ $r^2 = 0.024$

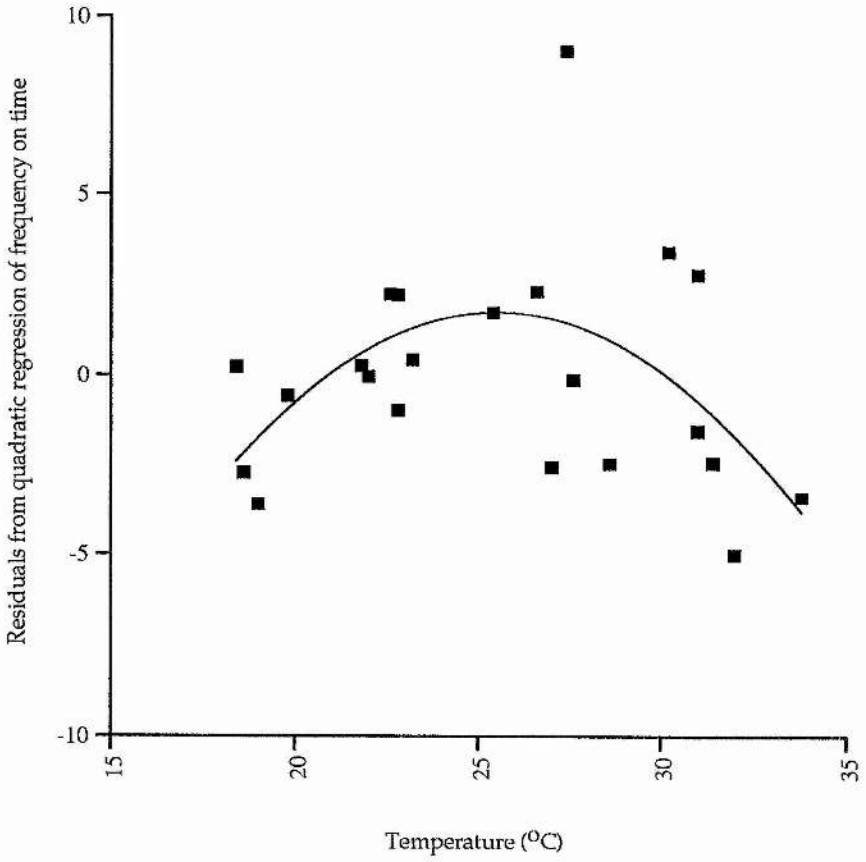


Fig. 7.13 Influence of temperature at flower bed on male *E. tenax* abundance on 14 August 1997 (time controlled for), $y = -0.081x^2 + 4.128x - 50.968$ $r^2 = 0.264$

light, their numbers have already decreased when temperature peaks, thus the apparent negative relationship.

D/ Influence of temperature on the hottest day

As already mentioned, 14 August was the hottest day of this study and observations done on this day were analysed separately. Figure 7.8b shows that the flies peaked in abundance before temperature reached its maximum. This suggests that above a certain temperature, these eristalines become thermally stressed. A quadratic regression on the overall data did not support this idea (T^2 factor not significant) possibly because only a few records were obtained at high temperatures in comparison to records at lower temperatures. Thus results from 14 August 1997 were analysed separately.

After having controlled for the effect of time, male *E. tenax* show a significant quadratic relationship with temperature ($n = 23$, $R^2 = 0.265$, T $p = 0.015$, T^2 $p=0.014$). A linear or quadratic relationship with light was also looked for but was not significant. Females, on the other hand, do not show any quadratic or linear relationship with temperature when time is controlled for (and even if time is not controlled for) ($n = 23$, $R^2 = 0.001$, T $p = 0.921$, T^2 $p = 0.920$). Figure 7.13 shows that the critical temperature above which *E. tenax* decrease in number (controlled for time of day, hence the residuals) is around 25°C.

Both male and female *E. pertinax* do not have a significant quadratic or linear relationship with temperature after time as been controlled for (males: $n = 23$, $R^2 = 0.07$, T $p = 0.230$, T^2 $p = 0.221$; females: $n = 23$, $R^2 = 0.106$, T $p = 0.149$, T^2 $p = 0.143$). Thus, they do not seem to become thermally stressed at these kinds of temperatures.

7.3.3 Handling time

Females and males of either species did not appear to have different flower handling times and so were analysed together. Observations were carried out on two days. One day (10.9.97) had a fairly constant humidity ranging from 53% to 58% but an ambient temperature varying from 14.5°C to 20.9 °C. The other day (2.9.97) was very overcast and humid (72 % RH, 16.2 °C). Only a few males of each species were seen at around 12.00 h. Their mean handling time provided a single observation per species.

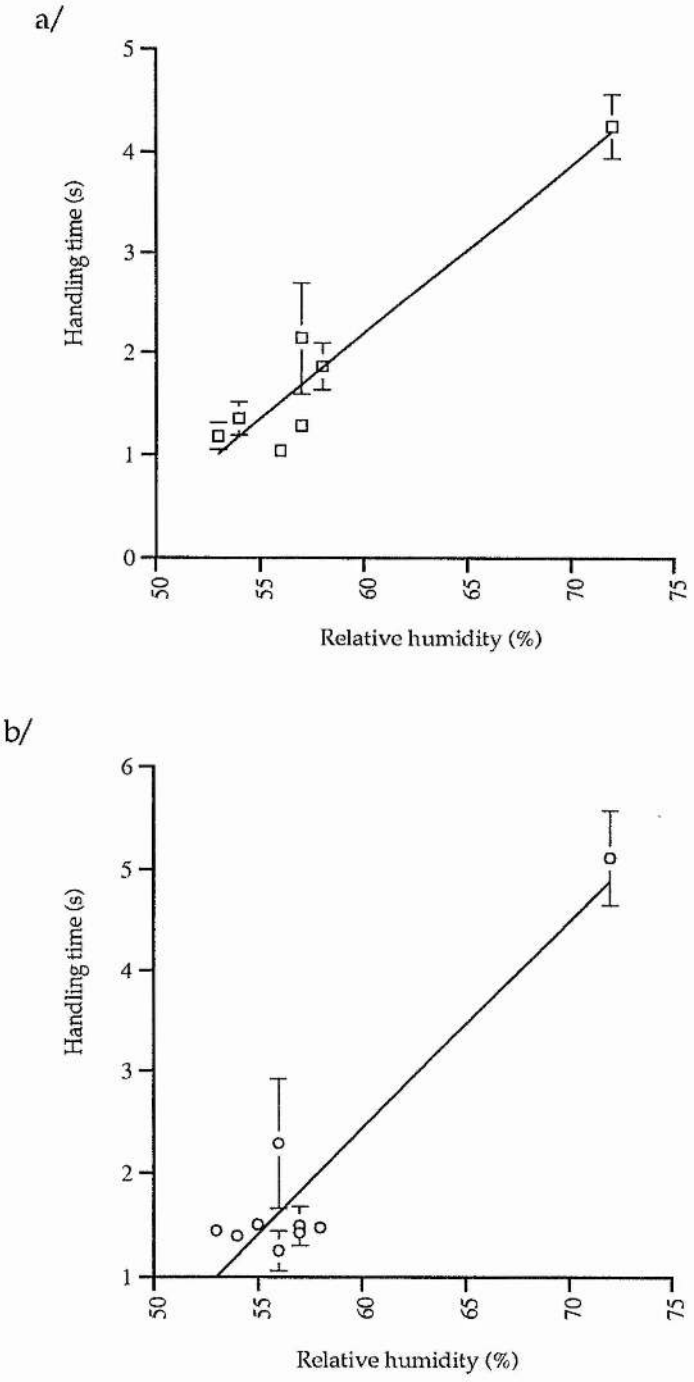


Fig. 7.14 Influence of humidity on flower handling time (oregano) on 2 and 10 September 1997

a/ *E. tenax*: $y = 0.168x - 7.903$ $r^2 = 0.913$
b/ *E. pertinax*: $y = 0.205x - 9.877$ $r^2 = 0.880$

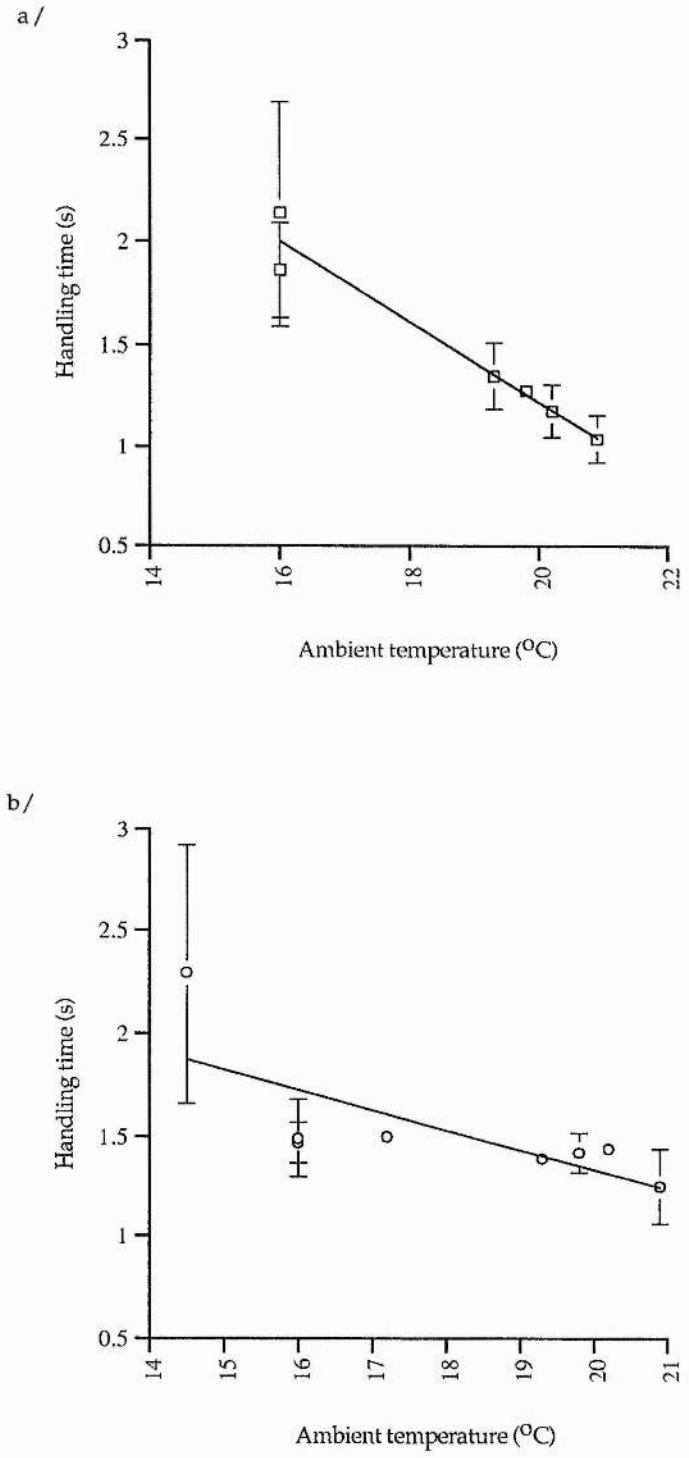


Fig. 7.15 Influence of temperature on flower handling time (oregano) on 10 September 1997

a/ *E. tenax*: $y = -0.195x + 5.115$ $r^2 = 0.957$
b/ *E. pertinax*: $y = -0.098x + 3.293$ $r^2 = 0.534$

Multiple regression analyses on the data from the two days suggest that the flower handling time of both species is strongly influenced by humidity (*E. tenax*: n = 7, R^2 = 0.956, RH p = 0.003, T_a p = 0.118; *E. pertinax*: n = 9, R^2 = 0.887, RH p = 0.001, T_a p= 0.589; Figure 7.14). Ambient temperature does not seem to have an effect on handling time. However, when the data from 10.9.97 are analysed on their own to eliminate the overwhelming effect of the high humidity on 2.9.97, temperature becomes a significant predictor of handling time (*E. tenax*: n = 6, R^2 = 0.958, T_a p = 0.007, RH p = 0.820; *E. pertinax*: n = 8, R^2 = 0.613, T_a p= 0.034, RH p = 0.359; Figure 7.15). Because of the low sample size, it was necessary to separate the data from the two sets to show the effect of these two climatic variables. Of course, it is not suggested that humidity itself influences handling time in these eristalines but rather that it is its effect on nectar volume and/or concentration that is important; volume/concentration and humidity are well correlated as was shown above.

7.4 Discussion

7.4.1 Hovering

The results here are in striking contrast with those of Wellington and Fitzpatrick (1981). Their description of the hovering behaviour for *E. tenax* does not apply to the present study. The males of this species were found to hover very little. This was also observed by Francis Gilbert (pers. comm.). However, male *E. pertinax* do behave in a similar fashion to the flies described by Wellington and Fitzpatrick. They hover in a territory with the same characteristics and do so for several minutes on a spot, frequently chasing passing insects. However, even though they keep station and return to the same position after a chase, it is not clear if male *E. pertinax* actively defend territories. Smith et al (1994) observed a similar behaviour in tabanid flies and suggested that interactions with other species could be an artefact from the chasing of potential mates. As with their tabanids, there is no evidence here that male *E. pertinax* display to females, or that females choose males. Wellington and Fitzpatrick (1981) also observed inspection of flowers by hovering male *E. tenax* and

confrontation with any insects on these flowers that were not conspecific females. Nothing like that was seen for *E. pertinax*. Nevertheless, when a male was removed (for measuring its thoracic temperature) its place was soon occupied by another male, suggesting that there is some competition for adequate spots. The present study did not look at the time budget of these males, and so it is not known if they also have a feeding and a resting territory. It is however dubious that Wellington and Fitzpatrick did manage to identify individuals with certainty over several days just by the abdominal coloration pattern. In addition, as was also observed by Wellington and Fitzpatrick (1981) for *E. tenax* in Canada, male *E. pertinax* do not usually hover in autumn.

Assuming that species identification is not the answer to these differences, there could be regional differences in the hovering behaviour of eristaline flies. If indeed *E. tenax* is common in the spring in Canada - whereas it is not very common in Scotland before the end of July - the described hovering pattern could be attributed to ecological factors related to the time of the year rather than to a species difference. It is possible that in the spring, the best mating strategy for these eristalines is to hover and to intercept passing females. One suggestion is that the density of flowers is lower at this season than later in the summer. It might be easier to locate females at a feeding "station" later in the summer. There is no evidence that females visit places where males hover in order to find a mate. This kind of behaviour has been reported by Alcock (1996) in the carpenter bee *Xylocopa varipuncta*. The males of this species aggregate and hover in spring afternoons. They release a pheromone that attracts females. However, in the present study, females were never seen to approach males. The anecdotal observation made in September, when a male *E. pertinax* resting on a leaf, chased little stones which were thrown by the experimenter could also represent an alternative mating strategy (perch-and-pursue) and would need to be studied further.

Hovering is a highly energetically demanding activity. It has been reported in several papers that hovering flies maintain a constant thoracic temperature. For example, Schutz and Gaugler (1992), found that in the tabanid *Tabanus nigrovittatus*, an early morning hovering group maintained their thoracic temperature at 28.3 ± 4.6 °C (ambient temperature range 15 - 22 °C), and the late morning group at 36.7 ± 0.78 °C

(T_a 22 - 28 °C). *Tabanus conterminus* hovered with a thoracic temperature of 35.2 ± 0.28 °C (T_a 18 - 27 °C). Females of these species do not thermoregulate as strongly as hovering males. Males did not exhibit any endothermy, and pre-flight warm-up was assumed to be strictly ectothermic. Also, Smith et al (1994) recorded a constant thoracic temperature of 40 °C, as much as 23.5 °C above ambient temperature, in the tabanid *Hybomitra arpadi*. It is certainly also the case for *E. pertinax* that hovering males maintain a constant high thoracic temperature (Chapter 5). This fly maintains its thoracic temperature around 30 °C, always well above the ambient temperatures recorded during this study (maximum ambient temperature 22.3 °C), but does not thermoregulate in forward flight. Therefore, it comes as no surprise that temperature has a strong influence on hovering duration. *E. pertinax* hover for longer periods when temperature is higher. However, they also hover for short periods as well. But at low temperature, only the short duration hovering bouts are observed. The lowest temperature at which *E. pertinax* was seen to hover was 12.0 °C. At this low temperature, hovering lasted only a few seconds after which the fly landed on a leaf and apparently proceeded with basking. It was seen in Chapter 5 that below 16°C, the flies do not manage to maintain a constant thoracic temperature of 30 °C. It is clear from Figure 7.3 that light cannot be responsible for the difference in hovering duration, because light levels were very similar on the two days which were studied in more details. However, temperature was different, being higher on 10 June. This is also confirmed by the regression analysis performed on the whole data set: light has no influence on the duration of hovering, but temperature has. In contrast, Fitzpatrick and Wellington (1983) suggest that for *E. tenax* and *Merodon equestris* light is more important than temperature for the hovering behaviour.

Ambient temperature was also found to have an effect on the proportion of hovering relative to resting. The proportion of hovering is highly positively correlated with temperature, i.e. male *E. pertinax* hover more than they rest as temperature increases. As a consequence of temperature being higher around midday, the proportion of hovering is the highest at that time and the lowest in the morning and afternoon. A very likely explanation for this is that it is easier for the males to maintain a high thoracic temperature when ambient temperature is also high. They

can thus hover more (and for longer). At lower temperature, they might have to stop more often to bask in order to rise their thoracic temperature. Endothermy could possibly be involved here as well, as at low ambient temperature (around 16 °C) the required thoracic temperature excess is around 14 °C. Smith et al (1994) also observed more frequent interruptions of hovering early in the morning in the tabanid *Hybomitra arpada* and suggested that this fly has problems maintaining its thoracic temperature at 40 °C.

There might be several reasons for the importance of a constant elevated thoracic temperature. While hovering, wing muscles are likely to produce a considerable amount of heat which probably helps keep the thoracic temperature high. However, thoracic temperature is not just elevated, but is controlled. This suggests that other advantages are associated with this high thoracic temperature. The high wing beat frequency required during hovering is likely to dictate a minimum thoracic temperature at which this behaviour becomes possible and to require some control over thoracic temperature. In addition, warmer males are probably more agile and are likely to fly faster to intercept passing insects. The competition that males possibly face in relation to their mating success might in part be the selective cause of the elevated thoracic temperature of hovering flies.

The time of day is also important, and mean maximum hovering duration has a significant quadratic relationship with time; this is not quite significant for the mean hovering duration probably because of the influence of the numerous short duration bouts. *E. pertinax* usually hover between 10.00 h and 17.00 h, with a peak around midday. The influence of the time of day means that even if temperature is high enough to hover early in the morning or late in the afternoon, the flies will not do so. This is clearly seen on Figure 7.3 : in the morning and at 17.00 h on 10 June, temperature was higher than the peak it reached on 3 June, but no males were hovering. *E. pertinax* show a peak of hovering around midday, but the duration of hovering depends on temperature.

It is possible that hovering is timed to correspond with activity, in particular feeding, in females. Such timing has been claimed by Alcock (1996) for the carpenter bee *Xylocopa varipuncta*: males' hovering activity is highly correlated with females' activity. Similarly, Maier and Waldbauer

(1979) found that males of the syrphids *Mallota posticata* and *Somula decora* patrol plants in the morning, when females are feeding, and wait in mating territories around rot holes (oviposition sites) in the afternoon at the time females come to oviposit (they mate repeatedly). As will be seen in the next section, female *E. pertinax* were observed to forage at similar times. In this species, timing could be linked to a diurnal pattern (importance of the time of day) and/or could result from climatic constraints such as temperature.

7.4.2 Foraging

In addition to *E. tenax* and *E. pertinax*, the oregano flower bed (and the aster) was used by honeybees, a few bumblebees and other medium-large eristalines. Not many interactions between the species were observed, so competition for food resources does not seem to be important. However, mate seeking by males was witnessed frequently.

In this type of study, it is difficulty to clearly identify the factors affecting insects' abundance, because all the climatic parameters are mutually dependent. It was found that these two eristalines are very much influenced by the time of day in their foraging pattern. They peak in number around midday. Obviously, temperature and light levels are very much linked to the time of day, so the flies could respond to either or both factors for foraging (as was suspected initially), but this does not seem to be the case. It became clear that something else than temperature and light was affecting the flies' abundance when looking at Figure 7.8. For example, on 8 August, temperature did not decrease towards the end of the afternoon but remained at around 24 °C. Light levels were sharply reduced by clouds from 15.00 h, and the number of *E. tenax* fell as well, possibly because of the decrease in light. However, on 20 August, *E. tenax* peaked at around 14.30 h, when light levels were quite low and similarly on 25 August. This is not as clear for *E. pertinax*, but is nevertheless supported by these and other observations. For example, on 19 September (the study done on aster), *E. pertinax* had reached high numbers at 10.30 h, when light was around 280 W m^{-2} and temperature was about 14 °C (Figure 7.10). Total numbers did not increase very much after that, even though light and temperature did so around midday. Also, in the afternoons of the four days when flies' abundance on oregano was

studied, *E. pertinax* numbers always decreased, even when light and temperature were high enough for these flies to forage. Gilbert (1985) also reports a decline in syrphids in the afternoon. The importance of time of day is also supported by observations on overcast days. During these days, very few flies are foraging, but they do so around midday. Interestingly on very overcast days, *E. pertinax* are more numerous than *E. tenax*; the former were seen on several occasions foraging in light rain. In addition to these observations, temperature and light do not explain much of the variation when these are the only factors used in the model to predict flies numbers (*E. tenax*: males $R^2 = 0.135$, females $R^2 = 0.073$; *E. pertinax*: males $R^2 = 0.184$, females $R^2 = 0.133$). Therefore, it was decided to investigate the effect of time of day. Indeed it came very significant for both species and increased substantially the variation explained by the model, in particular for *E. tenax*. Gilbert (1985) and Hovemeyer (1995) report that it is unlikely that syrphids measure the time of the day. Similarly, Willmer (1983) suggested that the pattern of abundance of insects on *Tilia* and *Heracleum* flowers was best explained by the effect of the weather, in relation to thermal constraints. Here, in particular for *E. tenax*, it seems that time of day is an important cue for foraging. Circadian rhythms have been demonstrated in many animals. For example, Moore et al (1998) showed that honey bees in the hive start by being arrhythmic but progressively become active and forage following a robust circadian pattern. Also, Meyerpeters (1993) found that the locomotor activity of the beetle *Carabus auronitens* follows a daily periodic pattern. This pattern is bimodal in early spring and becomes unimodal later in spring. However, it is not the only cue, and thermal considerations certainly play a part as well. Of course, the foraging pattern also depends on the plant and its position. Willmer (1983) showed a bimodal distribution of *Eristalis* on *Tilia* flowers and more of a single morning peak on *Heracleum* Thus, a particular pattern depends on the exposure to the climate and probably also on the nectar's characteristics. Variation in foraging patterns can reveal the constraints faced by insects.

In addition to the time of day, *E. pertinax* are very influenced by light levels. This is seen on Figures 7.8 and 7.9, and in particular on Figure 7.12. *E. pertinax* are more abundant when there is plenty of light. This seems contradictory to what had just been said about the foraging of this fly in

the rain, but only a few flies were observed in these circumstances. They still forage at low light (around midday), but in low numbers. On bright days, they also remain later in the field than *E. tenax* (e.g. on 14 August and other observations done in summer 1996, when *E. pertinax* were seen foraging until 18.00h as no more *E. tenax* were present). Therefore, foraging in *E. pertinax* is dependent on the time of day, peaking around 11.00 - 12.00 h; abundance is also related to light levels; and these flies can forage (in low number) in the rain and later in the afternoon.

When the whole data set was analysed, it seemed that foraging in *E. tenax* was only under the influence of the time of the day (temperature and light factors not significant predictors). However, looking at Figure 7.8, it is clear that the peak can be at any time between 10.30 h and 15.30 h, and it seems to correspond to the temperature peak. It is probable that the influence of temperature is not picked up by the model because at times when temperature remained high in the afternoon, as on 8 August, *E. tenax* decreased nevertheless. It looks as if the time of the day has a stricter control on the disappearance of *E. tenax* than on its appearance: by 16.30 h numbers have always fallen whatever the temperature. However, when only the data obtained before 14.00 h are considered, temperature has a strong effect on the males' abundance. The weak negative relationship between male *E. tenax* abundance and light is most probably an artefact. It is clear on Figure 7.9 that light peaks earlier than temperature. Thus, when light reaches its maximum level, male *E. tenax* are still increasing. When they reach their peak, light is already falling. Females do not seem influenced by temperature or light. The number of females usually remained rather low, without a definite peak, in comparison to males on these four days of observation, except on 25 August.

Therefore, these eristalines seem to respond to different cues when they forage. These cues make them more abundant around midday, but not at exactly the same time each day. The flies do not respond to all three cues at the same time: *E pertinax* use time of day and light; male *E. tenax*, time of day and temperature; female *E.tenax*, time of day only. Possibly using all three cues would give contradictory information, as light and temperature do not reach their maximum at the same time. For *E. pertinax*, temperature might not be very important. This smaller species does not thermoregulate in flight or while feeding (Chapter 5). It flies with its

thoracic temperature on average 2 °C above ambient temperature and is able to do so from ambient temperatures around 11 °C. Thus, the temperature encountered during this study would not constrain flight in this species. Light levels might be more useful, in conjunction with the time of the day, as an indicator of when to forage. *E. tenax* is the larger of the two species and thermoregulates in flight and when foraging (Chapter 5). For example, at 15 °C, it maintains its thoracic temperature about 7 °C above ambient temperature. It can achieve this higher thoracic temperature by endothermy and basking. While in flight, forced convective cooling has to be compensated for at low ambient temperature. Gilbert (1985) reports that *E. tenax* forages from an ambient temperature of 11 °C. Here the lowest recorded temperature was around 15 °C, reasonably in accordance with his findings. Therefore, at low ambient temperature *E. tenax* might have to use endothermic heat production if it is to fly and forage. This is energetically expensive and is possibly avoided. Thus, the increase in abundance with temperature could indicate that males wait until ambient temperature is adequate for flight to forage. But what about females? They follow a similar pattern to males, but usually remain in low numbers and do not seem influenced by temperature or light. It is possible that they do use endothermy more freely than males and so are less dependent on temperature for foraging. Being larger, they are at an advantage in that they are less affected by convective cooling when in flight. Also, they probably reach a higher temperature excess due to warm-up by solar radiation than males while foraging and basking. In addition, it seems likely that thermal constraints play a part in the foraging pattern of these flies because temperature at the flower bed rather than ambient temperature affects their behaviour in this study. This suggests that basking (on leaves of other plants at similar temperature) and the external heat gained while foraging are important for these foraging insects.

There are suggestions that even though the occurrence of male *E. tenax* is correlated with temperature, these flies can become thermally stressed if temperature gets too high. On 14 August, males peaked somewhat earlier than temperature and had decreased sharply by the time the temperature at the flowerbed had reached 30 °C. Males' abundance showed a quadratic relationship with temperature on that day (Figure

7.13). The model fitted for that particular day, suggests a critical temperature of around 25 °C but, according to field observations, this seems a bit low. Indeed on 25 August, there was no sign of decline when temperature reached 29 °C. A critical temperature around 29 °C seems more probable. This is in accordance with Kikuchi's (1965) findings. He observed that *E. tenax* appear from ambient temperatures ranging between 16 and 20 °C, reach their maximum abundance at 25-28 °C and decline in numbers at temperatures above 28 °C. Also, Willmer (1983) suggested that above a solar radiation of 600 W m^{-2} the number of insects (particularly large and of low reflectance, as *Eristalis* sp.) foraging on fully insolated *Heracleum* flowers decline. Here 700 W m^{-2} seems to be the level, well in accordance with her findings. It has to be noted that at such a solar radiation, nectar in *Heracleum* is crystalline, so insects might also become water stressed. Oregano's nectar was still fluid. In addition, Herrera (1990) describes *E. tenax* foraging in the morning (peak at 07.00-09.00 h) on *Lavendula latifolia* in southern Spain. The decline in numbers around midday supports the idea that *E. tenax* avoid overheating. In this study solar radiation was above 1100 W m^{-2} at midday (ambient temperature not given). Herrera (1990) also mentions that of the three syrphid species he studied, activity started at the same time regardless of body size, but large flies ceased to forage earlier than small ones at midday. According to the present results, *E. pertinax* is either less suceptible to overheating (smaller) or the effect of the high temperature was not picked up by the model because numbers had already fallen in response to decreasing light levels.

The timing of the peak in foraging means that these flies have very little nectar available per flower, usually of a concentration above 40%. Gilbert (1985) also suggests a peak of feeding (on nectar) at around midday for *E. tenax*. It is possible that a trade-off has to be taken between the amount of nectar available and the energy needed to go and get it. More would be available early in the morning but *E. tenax* would possibly have to spend more energy for thermoregulation to get it. Also this nectar would be quite dilute, so even more energy would have to be spent in carrying a big load of dilute nectar than a small amount of concentrated nectar later in the day. Sotavalta et al (1962) suggest that for *E. tenax* the preferred concentration of three sugar solutions (those sugars most commonly found in nectar) are: glucose 25-30%, fructose 40-50% and

sucrose 25-40%. These preferred concentrations correspond well to the concentration of oregano nectar that these eristalines get at their peak time. It is possible that their peak occurrence is timed so as to get nectar of the preferred concentration. Alternatively, it might just be coincidental that preferred nectar concentration coincides with peak abundance. Volume seems less important as the peaks correspond to low levels of nectar. However, these flies are certainly able to get nectar even when none could be sampled by the experimenter. So it is not possible to quantify the reward they get, but this reward seems adequate.

Female *E. tenax* were somewhat less numerous than males of the same species. The same, more striking, observation could be made for *E. pertinax*. However, female *E. pertinax* were more common than males on aster. Female and male *E. tenax* occurred in similar numbers on aster. In addition, *E. pertinax* was more numerous on aster than *E. tenax* (the same observation was made in 1996), but this was due to the increase in females number as males occur with similar abundance on both flowers. This could reflect a difference in diet. Both species take nectar and pollen (pers. obs.). This is confirmed for *E. tenax* by reports from Gilbert (1981 and 1985) and Herrera (1990). Gilbert (1981) found that female *E. tenax* take more pollen than males. No direct information is available for *E. pertinax*, but in general female hoverflies feed more on pollen than males. The nutrients are very probably needed for egg maturation. It is possible that aster offers more pollen than oregano (although this was not directly checked). Certainly, the flies were more often recorded taking pollen on aster than on oregano. So, one tentative suggestion for the difference in number of the two sexes of these hoverflies on oregano and aster is that females tend to be more numerous on aster because more pollen is available for them. In addition, Gilbert (1981) suggested that larger species take more nectar possibly because they need more energy. Female *E. tenax* might not need to take as much pollen as female *E. pertinax* because at that time of the year they have to prepare for overwintering and so have to store up energy reserves rather than mature egg. This is probably why, if aster offers more pollen than oregano, their numbers do not differ between the two foraging sites as strikingly as for *E. pertinax*. In contrast to *E. pertinax*, female *E. tenax* use both resources to the same extent. Also, it was also observed that *E. pertinax* were very common on a patch of mint very close

to the oregano flower bed. These mint plants have smaller flowers than oregano. As *E. pertinax* are smaller than *E. tenax*, it is likely that their proboscides are smaller as well (Gilbert 1981 found a relationship between proboscis length and size). It is possible that the lower occurrence of this species on oregano is related to it having more difficulties getting nectar out of these larger flowers. This might also be one of the reasons why *E. pertinax* peaks before *E. tenax* on oregano. It is possible that when the volume of nectar gets too low, *E. pertinax* cannot reach the nectar. A comparative study of proboscis length, food and flower preference (corolla length) could certainly be enlightening.

7.4.3 Handling time

Both humidity and ambient temperature influence the rate at which these eristalines sample flowers. *E. tenax* and *E. pertinax* seem to handle flowers at similar speeds. In this study, nectar availability was not measured but was assumed to be similar to that recorded during work on the flies' abundance. The present results suggest that at high humidity, when plenty of nectar is available, these eristalines spend more time per flower than when humidity is lower. This makes sense as the more nectar is available the longer the flies have to spend to collect it. In addition, this record at high humidity was done at an ambient temperature of 16.2 °C, a temperature at which the flies were observed to be still quite sluggish. They were probably not feeding and moving between flowers at their fastest speed. Indeed, the results also show a strong effect of temperature on handling time when humidity was fairly constant throughout one day. This suggests that as the flies get warmer they forage faster. However, it is not possible to disentangle this from the fact that nectar volume was certainly decreasing with temperature as a result of water evaporation and insect visitation. In addition, this rise in speed should be counterbalanced to some extent by the increase in viscosity of the nectar. Sotavalta et al (1962) showed that in flies drinking speed decreases with nectar concentration. Thus, as a result of their handling speed increasing with temperature, these eristalines forage faster around midday. This could be an advantage when temperature becomes so high that the risk of overheating is present. The time spent exposed while foraging might have to be limited (it could also be limited because of the risk of predation).

These results are only preliminary, and the effect of the various climatic factors and insect visitation could be disentangled to some extent with more data, in particular regarding nectar availability. By controlling for the latter, the effect of climate on the flies, in particular temperature, could be more easily identified. Thus, the influence of insect visitation and of the climate on nectar would be controlled for. However, it is difficult to measure nectar volume accurately. It was obvious during this study that even when nectar cannot be sampled by capillary tubes, some is available to insects. The variation of nectar volume in this situation is impossible to determine. The identification of a plant foraged upon by these eristalines and producing more substantial amounts of nectar could be of great help.

This chapter has demonstrated the importance of both the time of day and climatic factors (temperature, light and relative humidity) on various activities of *E. tenax* and *E. pertinax*.

Hovering, a mating strategy, is exhibited by male *E. pertinax* in the early summer, but not usually later in the season. In contrast to some reports, Scottish male *E. tenax* were not seen hovering to any extent comparable to *E. pertinax*. In *E. pertinax*, the timing of the hovering behaviour seems to respond to the time of day. The duration of hovering bouts is strongly influenced by ambient temperature. This was expected as a constant elevated thoracic temperature is maintained during this activity.

Foraging, in both species, is also related to the time of day, and the flies peak in number around midday. This timing corresponds to the availability of nectar at the preferred concentration, but this might be coincidental. In fact, abundance is also under the influence of temperature (at the flower-bed) in male *E. tenax*, and of solar radiation in *E. pertinax*. The importance of these two factors was related to the thermal constraints faced by these eristalines and possibly to the trade-off faced by these flies in obtaining adequate food rewards. However, it is not possible to separate these factors clearly, because all of them are mutually dependent. The relative abundance of the two sexes and the two species on two flowers was tentatively explained by diet differences.

These two eristalines handle flowers at a similar speed, which is dependent on the relative humidity of the air and on ambient temperature. The influence of humidity is certainly exerted through nectar availability and concentration rather than directly. Temperature has an even more complex effect as it is correlated with the flies' agility, the flies' abundance (hence depletion of nectar volume) and the characteristics (volume and concentration) of the nectar.

Chapter 8 - Conclusions and new directions

8.1 Introduction

This last chapter summarises and links together the main results of this thesis. The dominant themes, such as the importance of size, aspects of water balance, thermoregulation, overwintering, hovering and foraging, are discussed in more depth; and further investigations which it would be worthwhile attempting to deepen our understanding of the biology of *E. tenax* and *E. pertinax* are suggested.

8.2 The importance of size

Chapter 2 showed that it is important to consider several size factors in studies investigating the effect of size the biology of an organism. Mass, although readily determined, is not necessarily the best or only factor to use. Mass can vary markedly within individuals, depending on the feeding and reproductive state. The fact that the relationship between various size factors is logarithmic in these eristalines means that in large flies, individuals with a similar thoracic width can have very different masses. Thus, linear dimensions should also be considered. Using several factors can reveal sexual dimorphism. For example, in *E. pertinax* females are heavier than males but of the same shape. However, the two sexes are of different shape in *E. tenax*. In this project, mass and thoracic width were the two factors used.

It was shown throughout this work that sometimes one size factor is not a significant predictor of the studied variable (such as warm-up rate, stable flight temperature, etc.) but another one is. It is thus recommended

to use both size factors, and eliminate one if not significant. Using both size factors also allows one to distinguish "real" sex effects from sex effects due to size difference. This raises some concerns about studies which have used only one size factor, and about the suitability of the data collected in the field. Quite often in the field a single size factor, usually mass, is recorded, but whenever possible a linear dimension should be obtained as well.

In general, the expected influence of size on the many variables investigated have been supported by the present work. In particular, large/heavy flies exchange heat more slowly with the environment, have faster endothermic warm-up rates (*E. tenax*), have higher voluntary flight temperature (at low ambient temperature) and maintain larger temperature excesses in flight than small/light ones. For water losses, it is difficult to predict how size should influence the rate of water loss because two avenues for the losses have to be considered. Cuticular water losses are probably related to the surface area, but it is not certain how respiratory losses are affected by size. Nevertheless, a positive relationship between water loss rate and size could be found in some instances (mostly in live flies).

What are the implications for the flies? Large flies should attain a larger temperature excess when basking and warming up endothermically, but should take longer to reach it. But large flies will have to raise their thoracic temperature on cool days to be able to fly. So large flies can forage when it is too cold for small ones to do so, but they are constrained to reach a higher thoracic temperature to fly. So they probably will only spend the energy necessary to warm up if food rewards are likely to compensate for the expenditure. This is probably why *E. pertinax* were sometimes seen foraging on very overcast days and even in light rain. They do not need to raise their thoracic temperature much higher than ambient. Much lighter syrphids, such as *Episyrphus balteatus*, were frequently observed foraging in similar weather and in early evening. *E. tenax*, on the other hand, would have had to warm up endothermically to forage in such conditions, an expense they seem to avoid. Large flies are also more prone to overheating. It was shown that *E. tenax* numbers fall when the temperature at the flower-bed reaches 28 °C.

Further studies should investigate more fully the effect of size on the ecology to these eristalines. For example, the size of foraging flies should be measured throughout the day and related to hygrothermal constraints. Similarly, the effect of size on hovering *E. pertinax* should be investigated as large males might be at an advantage for hovering at low ambient temperature.

Size has been a recurrent theme throughout this thesis and it has been demonstrated that it affects most of the aspects of life of these eristalines.

8.3 Water balance

The problems associated with the various units used by different authors were discussed. The units used to express rates of water loss have been recently harmonised (Hadley 1994). Units referring to cuticular permeability should be used when dealing with resting insects, when respiratory losses are low. When respiratory losses become a substantial proportion of water losses, such as in active animals, the units for total water loss (rather than for cuticular permeability) should be used. In the present work both kind of units had to be used in order to compare the results with the published literature.

The dangers of restraining insects in order to obtain continuous records of their mass loss have been highlighted here. Successive weighings of unrestrained individuals may increase the rate of water loss because of the stress caused, but restraining them may reduce the measured rate because of the creation of a humidity boundary layer and/or of condensation of water on the netting material. Nevertheless, the gravimetric method is appealing because of its simplicity and is still very useful, albeit used with due care. An initial and a final weighing of otherwise unrestrained insects can get round much of the difficulties.

This method allowed the determination of the rate of water loss in *E. tenax* and *E. pertinax* in conditions close to those encountered in the field. These flies' rates of water loss fall within the range of those for other mesic insects, and in particular Diptera, but are in the upper part of the range.

Thus, these eristalines do not seem particularly good at controlling their water losses, and this might indicate that lack of water may not be a problem for them, at least in summer. However, rather better a control (reduced water loss rates) was identified in two situations. Overwintering *E. tenax* do face water stress and were shown to reduce their water losses. In association with this physiological adaptation to water stress, these flies select a very humid microhabitat to overwinter in order to reduce water losses. Nevertheless, they do become dehydrated. Also, male *E. pertinax*, which are even less likely to be water stressed than females as they feed mainly on nectar and produce a substantial amount of metabolic water through flying and hovering, lose water faster than females, except in the most desiccating conditions. It has frequently been observed that males extrude water from the anus while hovering. This suggests they actually have a water surplus. In addition, the control of water losses was inferred in Chapter 4, where it was shown that the thoracic temperature at which the flies equilibrate to (which depends on evaporative cooling) is regulated to some extent. These results lead to the suggestion that these flies are able to reduce their water losses, but do so only when water becomes a critical factor. The crude methodology used here did not allow the direct measurement of the regulation of water losses by flies placed in conditions of varying temperature and humidity.

E. tenax was found to lose water faster than *E. pertinax*. Possibly, *E. tenax* do not need to control their water losses to the same extent as *E. pertinax* because, being larger, they can carry bigger water reserves. Another explanation, which is supported by data from thermal investigations and ecological studies, is that a higher rate of water loss might protect *E. tenax*, to some extent, from overheating. These flies were seen to decrease in numbers when temperature at the foraging site rose above 29 °C (confirming findings from Kikuchi 1965). Also in Chapter 4 it was shown that due to the higher evaporative cooling, warm-up is slower and cooling down faster than in *E. pertinax*. It is hypothesised that in summer in Scotland, *E. tenax* are more likely to be at risk of overheating than of dehydration and might use evaporative cooling to lower this risk.

There are many avenues that would be worth pursuing about the water balance of these flies. First, a more accurate method should be used to compare rates of water loss in different conditions of temperature and

humidity. The main problem here was that the high humidity was disturbed when the pots were opened and took some time to be restored. Possibly, the use of a hermetic chamber in which the flies could be left unrestrained and the climate controlled externally would be adequate. Electronic moisture sensing would certainly provide the information sought. Alternatively, the attempt to use radioisotopes should be pursued. The problem of not being able to withdraw any haemolymph could be addressed by letting the flies ingest tritiated water from food or take it up from an atmosphere in equilibrium with tritiated solution. The flies could then be kept in particular climatic conditions, and after a chosen period, the radioactivity of their body would be determined. It might be possible to replace female *E. tenax* in their overwintering habitat for this experiment, although the disturbance might be too much and the flies might not stay in. These kind of experiments would allow a more accurate determination of the rate of water loss and would permit to confirm that the flies reduce the rate at which they lose water in certain conditions.

However, the problem of increased activity at high ambient temperature would need to be addressed. A similar method to that of Nicolson and Louw (1982) with carpenter bees would certainly shed a great deal of light. They coupled O_2 consumption measurement to electronic moisture sensing. This allowed them to show that these bees remain in water balance in flight (below 27 °C) because of metabolic water production. By showing to what extent *E. tenax* and *E. pertinax* rely on metabolic water production and coupling this with ecological observations, it could be confirmed that these flies might not be water stressed in Scotland in summer.

On a larger scale, water balance in these eristalines should be compared with that of conspecifics in more arid regions. For example, these flies are common in places like Israel (Simon Potts pers. comm.). In such a climate, water is more likely to be a rare resource than in Scotland, and the flies might have to reduce their water losses. If this were the case, it would confirm that Scottish eristalines are rather "leaky" because they do not need to control their losses. However, if these flies can obtain enough water from nectar and metabolic water production (essentially during flight) in more arid parts of the world, their rate of water loss might not be lower than in Scotland. In fact, it could be an advantage to be

somewhat "leaky" (if control can be exerted) in hot places, as evaporative cooling can help prevent overheating. However, there are only a few examples of insects using evaporative cooling (e.g. honeybees (Heinrich 1980a, b) and the desert cicada *Diceroprocta apache* (Toolson 1987, Hadley et al 1989)), because usually water is a scarce resource.

With more insight into the limits of these eristalines at controlling their water losses, and also with reference to the lower losses of overwintering female *E. tenax*, it would then be necessary to investigate the physiological processes involved. This would require the use of various sophisticated techniques. For example, the lipid composition of the cuticle would have to be determined and compared for summer and overwintering flies. The transpiration rate through a piece of excised cuticle could also be measured (Hadley 1994). In addition, overwintering resting flies might control their respiratory water losses. This possibility could be investigated using the electronic moisture sensing technique while additionally recording the carbon dioxide production (as described in Hadley and Quinlan 1982).

It is therefore clear that the present work has opened the door to numerous experiments relating to the water balance of these eristalines, and in particular of *E. tenax* which has to cope with the very contrasting climatic conditions of the summer and winter. The availability of this fly both locally and across varied climates makes it an ideal subject for such investigations.

8.4 Thermal biology

The problem of working with "leaky" flies was discussed at length in relation to Bakken's suggestions (1976). Essentially, it was shown that using the temperature at which the insects equilibrate rather than ambient temperature leads to more accurate estimates of cooling and warming constants, but care should be taken that the depression in temperature is the result of passive evaporative cooling rather than active evaporative cooling in response to overheating. In the latter case, cooling down is actively accelerated and thus is not part of a passive rate of temperature

change. The main effect of evaporative cooling is that these flies warm up more slowly than they cool down. The present work is one of the only studies that has followed Bakken's recommendations of using equilibrium temperature rather than ambient temperature to calculate cooling and warming constants.

The endothermic capabilities of these two eristalines were demonstrated in Chapter 5. *E. tenax* of similar size to *E. pertinax* warm up faster endothermically than *E. pertinax*. Both species regulate the thoracic temperature at which they take off, and *E. pertinax* tend to take off at lower thoracic temperatures than *E. tenax*. The drone fly is a moderate thermoregulator in flight and while feeding on flowers, but *E. pertinax* is not. However, the latter maintains a constant elevated thoracic temperature (at 30 °C) while hovering. By using a very fine thermocouple, it was possible to measure the changes in thoracic temperature during warm-up, at take-off, and essentially during "free" flight and after landing. This technique allowed the confirmation of the validity of the "grab and stab" method (for these flies) to estimate body temperatures of free-ranging insects. In particular, these eristalines do not seem to warm up endothermically after being netted, in contrast to of the bees Stone and Willmer (1989b) investigated; and thoracic temperatures estimated by the "grab and stab" method are in good accordance with those measured with the insects attached, to a thermocouple, but flying freely.

The possibility that *E. tenax* thermoregulate by shunting hot haemolymph from the thorax to the abdomen where it can lose heat was investigated by implanting fine thermocouples in the thorax and abdomen and continuously recording temperature changes. Haemolymph shunting is apparently in operation, and helps the flies cool down, but it is unlikely that this is a controlled process.

Are these thermoregulatory abilities used in the field? Endothermic warm-up is certainly necessary before flight for female *E. tenax* that leave their overwintering site in early spring. These flies have no other means to raise their thoracic temperature high enough to support flight. In summer, flies have access to solar radiation and can raise their body temperature by basking. Flies were frequently seen basking in the field. Endothermy frees insects from thermal constraints and allows them to exploit food resources that are inaccessible to other insects. However, endothermic warm-up

being energetically costly, it seems likely that it should be used only if cheaper means are unavailable, unless the cost can be offset by the gain of greater rewards. *E. tenax* thermoregulate and on cool days will need to raise their body temperature before flight. To what extent they use endothermy could not be determined with certainty. The records of abundance of foraging drone flies do not suggest that the flies exploit their endothermic abilities to forage early when more nectar is available as bumblebees do (Willmer 1983). Males increase in number with temperature (up to a critical temperature around 28 °C) which suggests that they mainly rely on solar heat. Females seem to forage independently of temperature (once the time of day has been controlled for) which suggests that they might use their endothermic abilities to some extent. Their larger size might give them an advantage over males with regard to convective cooling. *E. pertinax* also forage independently of temperature, but in contrast with female *E. tenax* their abundance is correlated with solar radiation. In addition, they do not thermoregulate and are capable of flying with thoracic temperatures equal to most of the ambient temperatures recorded in the field. They most likely bask before flying when temperature is low, but they probably do not usually use their endothermic abilities. Indeed, males of this species were sighted several times foraging in very overcast situations and even in light rain, suggesting they do not need to elevate their thoracic temperature much above ambient (as was checked by "grab and stab' in the laboratory). Being small in mass, probably allows them to fly with low thoracic temperature (low wing loading). The comparison of the "grab and stab" data obtained in the field and in the laboratory shows beautifully the influence of solar radiation on body temperature in flying flies (Figures 5.14 and 5.16). At the same ambient temperature, body temperature is always a few degrees (about 3 for *E. tenax* and 2 for *E. pertinax*) higher in the field. The extra heat comes from solar radiation.

It is possible that *E. pertinax* rely on endothermy prior to hovering as they are unlikely to reach the high necessary thoracic temperature by basking alone when temperature and solar radiation are low. Indeed, when ambient temperature is between 12 and 16 °C, males have difficulties keeping their temperature high enough and they only hover for a few seconds. They nevertheless have a thoracic temperature about 10

°C above ambient, which they undoubtedly reach by a combination of endothermy and basking.

Hovering is related to the reproductive strategy, and there is considerable competition between males to mate with females. This might be the reason why energy is spent to raise thoracic temperature. There might have been a strong selection pressure for thoracic temperature to be kept constant and elevated. Such thermoregulation has been observed in other hovering flies such as tabanids (e.g. Schutz and Gaugler 1992, Smith et al 1994). Thus, this is not unique to *E. pertinax* and suggests some selection pressure at work on this behaviour. Partly, such a high temperature might be a pre-requisite for hovering. To remain airborne in one spot demands a high muscle power. The activity of the flight muscles in turn produces heat. In the light of the present results, it seems that the high thoracic temperature is needed in order to hover rather than being selected for in order to compete with other males (for example by being more agile or faster to intercept females). That this is the case is supported by the fact that if ambient temperature is too low, *E. pertinax* attempt to hover but can only do so for a few seconds. Thus, it seems that the muscles require such a high temperature to keep the fly airborne. However, the energy involved in raising thoracic temperature might be spent because of the competition between males for mating. If no hovering means no mating, the pressure is such that the energy will be spent. However, Schutz and Gaugler (1992) found that males of the same species of tabanids (*Tabanus nigrovittatus*) had become adapted to two different thermal regimes, effectively separating them into two groups hovering at different times. Schutz and Gaugler (1992) suggest that such a separation might serve as a prezygotic isolating mechanism between this species and its sibling species (*T. conterminus*) which hovers between the periods used by *T. nigrovittatus*. This would prevent interferences between males. However, they also propose that this division in hovering period could reflect alternative strategies for dealing with the constraints of hovering. Thus, there seems to be some plasticity in the selection of the thoracic temperature while hovering in these tabanids.

The issue of using endothermic and thermoregulatory abilities for some activities but not others shows that energy expenditure is prioritised. High costs have to be compensated for by high rewards, and the

competition involved in getting these rewards is possibly the source of the pressure that makes the insects use or not use their abilities. It is suspected here that competition for food is not very high, and *E. pertinax* get sufficient food reward without needing to spend energy on endothermy and thermoregulation (although they are capable of it). However, where mating is concerned, the reward is probably worth the expense. It would be very interesting to extend such investigations to other hovering insects to see if the same pattern is repeated. The thoracic temperature during hovering in relation to wing load should also be investigated. It would be expected that if the selected temperature comes from the muscle physiological constraints, this temperature should be higher in insects with a higher wing load.

8.5 Behaviour and ecology

8.5.1 Overwintering

The work done for this thesis is a preliminary study from which many questions arose that would invite further investigations.

The sites selected by female *E. tenax* to overwinter provide a stable warm and very humid (> 90% RH) microclimate. The flies are protected from the large and rapid climatic variations that occur outside. The most popular sites identified were caves, old mine shafts and buildings in ruin.

The exploitation of such a microclimate helps the flies survive the coldest period of the winter. It may or may not be associated with physiological adaptation to overwintering. For example, it was shown that in addition to selecting a very humid site, the flies physiologically lower their water losses. What about the risk of freezing? From the evidence gathered, the temperature in the crevices remained well above freezing, so the flies would not need any special adaptation to deal with this problem. However, this might not be the case in colder regions. Siuda (1963) showed that in Poland the temperature in overwintering caves fell below freezing. In this situation, the flies have to deal with the possibility of freezing. They could produce anti-freeze compounds for example (e.g. Zachariassen 1985, Davenport 1992). A detailed study of the physiological

processes involved in response to the risk of freezing could be worthwhile.

Clustering might also be exploited. A cluster reduces the effective surface area of the flies. Clusters were only seen in some crevices, whereas in others, the flies were spaced out when there was more than one occupier. To find the significance of clusters fine probes would have to be placed in and out of clusters to measure humidity and temperature and see if gradients exist. In addition, the microclimate provided by the crevices where clusters are found should be compared to that of crevices where flies do not cluster together. For examples, the former might be less humid or more susceptible to temperature variation. These tests would be easy to carry out as long as a site could be selected that would not be disturbed by outsiders, and they would provide interesting insight on the behaviour of crevice selection. A similar comparative study of occupied and unoccupied crevices would shed light on the cues these flies use to select crevices.

The present work suggests that female *E. tenax* start and end overwintering in response to changes in temperature: in autumn temperature falls, and the flies look for overwintering sites; in spring temperature rises, and the flies leave the sites. This would need to be confirmed with records covering several years. In addition, Siuda (1963) and Moog and Ernst (1978) hypothesised that light (photoperiod changes) could also be an important cue. Indeed, it seems that crevices close to openings are preferred. Altering the light that crevices receive would be an easy way to test for any influence of the photoperiod.

Movement of flies also needs to be investigated. This requires frequent visits to the site and recording of flies' number and position. Ideally, an acceptable way of marking the flies should be developed. This should not disturb them too much so that they would not fly away.

Movement within crevices would reveal responses by the flies to changes in climate. For example, it was often noted that the flies were at times very deep in crevices and at other times closer to the opening. Movement deep in the crevices could be in response to colder and/or drier weather. Obviously, before making any conclusion it would have to be ascertained that thermal and humidity (already shown here) gradients exist in the crevices.

Movement in and out crevices could give insight on emigration/immigration. Flies might get out to collect water and might come back to the same or another overwintering site. Putting netting at the openings of a site could catch flies attempting to leave, and would confirm that disappearances are due to flies leaving rather than flies being predated upon. If they do get out, the caught flies could be weighed, measured, their fat reserves and their hydration state estimated. With such data, suggestions could be made on the reason for such trips. For example, some flies might get so dehydrated that they do not have any other choice than to go an collect water, even though this would lower their energy reserves. From the present results, immigration does not seem very common.

This issue of *E. tenax* being active in winter needs to be addressed in more depth. There are various reports of flies seen outside in winter (e.g. Hastings 1988, Kato 1943, Siuda 1963) but no such observations were done during this work. Apart from Siuda's record (in Poland) the others are from less harsh regions than Scotland. A comparative study of overwintering behaviour in various regions would be most interesting. It is possible that where flowers are available, and temperatures do not fall as low as in Scotland, *E. tenax* has a shorter overwintering period (or maybe none at all). Perhaps in such regions the flies come out from the overwintering site and return if the weather deteriorates. In any case, such a behaviour would only be possible if food is readily available.

This part of the project gives rise to more questions than it answers, but could lead to some stimulating research on the behaviour and physiology of overwintering of *E. tenax* and possibly on comparative studies on the biology of this fly during the winter months in various parts of the world.

8.5.2 Hovering

There is controversy about the hovering behaviour of these eristalines. On the one hand Wellington and Fitzpatrick (1983) claim that *E. tenax* in Canada defend a territory and display hovering behaviour commonly in spring but not in autumn. In contrast, the present work suggests that in Britain hovering is not a common behaviour of *E. tenax*. *E. tenax* seem rather to search for mates at flowers. However, male *E. pertinax*

do hover for very long periods in spring, but not in autumn. The defence of a territory was not investigated in great depth, and this would need to be done.

It is clear that hovering behaviour of these eristalines needs to be investigated much more thoroughly. First, a comparative study of the hovering behaviour of *E. tenax* needs to be undertaken to identify differences between various parts of the world. If such differences are confirmed, the underlying factors should be explored. They might be linked to the abundance or distribution of foraging and/or ovipositing sites used by females. The switch in mating strategy mentioned by Wellington and Fitzpatrick (1983) for *E. tenax* (from hovering in spring to not hovering in autumn) and observed here for *E. pertinax* suggests the influence of such a factor.

A comparative study across many parts of the world should be undertaken to clarify the factors that influence hovering in these eristalines.

8.5.3 Foraging

Quite a few studies on pollination ecology (Herrera 1988, 1990), on hygrothermal constraints faced by foraging insects (Willmer 1983, Willmer and Unwin 1981) and on ecology in general (Gilbert 1981, 1985) have involved these eristalines. This project was more concerned with the hygrothermal aspects associated with foraging, and these have been discussed in the thermal biology section of this chapter. Again, this work could be extended to regions offering contrasting climates to see how the flies are affected by these climates and how they alter their behaviour. Also, flower preference by males and females could be investigated. Do females forage more on flowers providing more pollen and males concentrate on nectar offering flowers?

As a last word, it is therefore suggested that a comparative study of the environmental physiology and behaviour of *E. tenax* and *E. pertinax* and other syrphids should be carried out in regions offering contrasting climates. Several such studies have been done on insects. For example,

Ritland (1998) showed that the mimetic butterfly (*Limenitis archippus*) has a clinal variation in wing colour that is linked to the distribution of its mimicry models. Also, Holloway (1993) found a trend between temperature and colour pattern in several species of the genus *Eristalis*. He reports that paler insects are more abundant in warmer months and that female *E. arbustorum* are paler inland than in coastal areas. Willmer and Stone (1997) conducted another stimulating study across a broad geographical range with bees. They conclude that desert bees are (primitively at least) adapted to cold desert dawns and dusks rather than to the heat of the middle of the day, and so are active early and late in the day. They also propose that the evolution of endothermy in desert bees having to forage during the cold periods of the day may have acted as a pre-adaptation for the invasion of temperate regions. Hoverflies, because of their world wide distribution, offer a similar opportunity to investigate the evolution of adaptations to hygrothermal constraints.

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